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Evaluation of Five Jet Fuels in the *Salmonella-Escherichia coli* / Microsome Plate Incorporation Assay

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14. ABSTRACT
Five jet fuels were evaluated for mutagenic activity in the *Salmonella typhimurium-Escherichia coli*/microsome plate incorporation assay. The assay was performed using the plate incorporation procedure with *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and *E. coli* strain WP2 (*uvr*A) in both the presence and absence of a metabolic activation (MA) mixture containing an Aroclor 1254 induced rat-liver S9. A range-finding experiment and two metabolic investigations, using first five and then ten percent S9 fraction, were conducted. In the second and conclusive experiment for mutagenicity, dose levels for R-8, R-8 from algae, S-8 and Swedish Biofuel consisted of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate. The doses for Amyris were 0.0013, 0.0025, 0.005, 0.01, 0.02, 0.039, and 0.078 µl/plate for the four Salmonella strains and 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate for the *E. coli* strain. The five jet fuels, Amyris, R-8, R-8 from algae, Fischer Tropsch fuel S-8, and Swedish Biofuel, were judged to be nonmutagenic under the test conditions used in this experiment; therefore, the test substances were determined to be negative in the bacterial reverse mutation assay.

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PREFACE

Funding for this study was part of “Stimulus Funding to AFRL/RZ in support of Toxicology Assessment of Biomass Aviation Fuel.” The Technical manager for the Air Force Research Laboratory/Propulsion Directorate, Fuels Branch (AFRL/RZPF) was Timothy Edwards, PhD.

This research was conducted under the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. (HJF) contract, FA8650-05-2-6518. The program manager for the contract was Mark Hoffman of the Air Force Research Laboratory, 711 Human Performance Wing, Human Effectiveness Directorate, Biosciences and Performance Division (711 HPW/RHP). The technical manager for the project was Dr David Mattie in the Applied Biotechnology Branch of 711 HPW/RHP who served as the Sponsor Representative for this study under Good Laboratory Practice Standards.

The authors want to acknowledge the Alternative Fuels Certification Office (AFMC 77 AESW/LF) for their support of the toxicity research program for alternative fuels in 711 HPW/RHP and Teresa R. Sterner of HJF for assistance in the preparation of this technical report.

The study was conducted in compliance with the United States Environmental Protection Agency Good Laboratory Practice Standards, 40 CFR Part 792, with the following exception: Characterization of the test substances (identity, purity, and stability) and the supporting analytical chemistry of the dose formulations were not provided to the testing facility as this testing was not performed by the Sponsor.

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1.0 EXECUTIVE SUMMARY

Five jet fuels were evaluated for mutagenic activity in the *Salmonella typhimurium-Escherichia coli*/microsome plate incorporation assay. The assay was performed using the plate incorporation procedure with *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 and *E. coli* strain WP2 (*uvr*A) in both the presence and absence of a metabolic activation (MA) mixture containing an Aroclor 1254 induced rat-liver S9.

The range-finding experiment was conducted with the five test substances with strain TA100 over doses of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate (100 µl) in the presence and absence of MA containing five percent S9. The first experiment for mutagenicity was conducted with the five tester strains in the presence and absence of MA containing five percent S9. Doses for R-8, R-8 from algae, Fischer Tropsch fuel S-8 (S-8), and Swedish Biofuel consisted of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate. Due to cytotoxicity observed in the range-finding experiment, doses for Amyris were lowered to 0.005, 0.01, 0.02, 0.039, 0.078, and 0.156 µl/plate. However, since only two nontoxic dose levels were achieved with strains TA1535 and TA98 in the absence of MA, this portion of the experiment was retested over lower doses ranging from 0.0013 to 0.039 µl/plate. No statistically significant increase in the number of revertant colonies was observed with any of the test substances, except for a slight increase seen with S-8 and TA1535 in the presence of MA which was statistically significant ($p < 0.01$) by regression analysis; however, it was so slight it was not considered to be a mutagenic response or biologically relevant. Cytotoxicity was only seen with Amyris and Swedish Biofuel under certain test conditions.

In the second experiment for mutagenicity, dose levels for R-8, R-8 from algae, S-8 and Swedish Biofuel consisted of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate. The doses for Amyris were 0.0013, 0.0025, 0.005, 0.01, 0.02, 0.039, and 0.078 µl/plate for the four *Salmonella* strains and 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate for the *E. coli* strain. The test substances were evaluated with MA containing ten percent S9. No statistically significant increase in the number of revertant colonies was observed with any of the test substances, except for a slight increase seen with R-8 and TA1537 in the absence of MA which was considered statistically significant ($p < 0.01$) by regression analysis; however, these increases were not reproducible and were not considered to be a mutagenic response or biologically relevant. Cytotoxicity was only evident with Amyris and Swedish Biofuel under certain test conditions.

Amyris, R-8, R-8 from algae, Fischer Tropsch fuel S-8, and Swedish Biofuel were judged to be nonmutagenic under the test conditions used in this study; therefore, the test substances were determined to be negative in the bacterial reverse mutation assay.

2.0 INTRODUCTION

The US Air Force is pursuing the development of alternative fuels to decrease dependence on foreign oil sources. Alternative fuels need to be examined for potential hazards to Air Force personnel. The 711 Human Performance Wing, Human Effectiveness Directorate, Biosciences and Performance Division, Applied Biotechnology Branch (711 HPW/RHPB) conducted a toxicology program for a Fischer Tropsch (FT) fuel (S-8), the first alternative jet fuel to be certified for use in the U.S. Air Force fleet. The data from this first alternative fuel, as well as the database for JP-8 jet fuel, will be the baselines for comparing future bio-based alternative fuels. There are already many bio-based jet fuels that need to be examined for potential toxicity as they undergo further development. A toxicology research program for bio-based fuels was developed with mutagenicity testing as one of the primary studies to conduct. Microbial mutagenicity assays are capable of rapidly detecting the mutagenic activity of many materials, including a wide range of chemical classes. Many chemicals that elicit a mutagenic response in the *Salmonella* assay have been shown to be potentially mutagenic and carcinogenic to humans and laboratory animals (Zeiger, 1998). One advantage of using the procedure with *E. coli* is that this strain has an A-T base-pair at the critical mutation site and thus is sensitive to some agents that are not detected by the *Salmonella* strains (Mortelmans and Riccio, 2000). Because microbial mutagenicity assays are short term, sensitive, and reliable tests for assessing mutagenic potential, their use for genotoxic evaluation of chemicals is appropriate (Mortelmans and Zeiger, 2000).

The *Salmonella* tester strains have mutations in the histidine operon, a mutation that leads to a defective lipopolysaccharide coat (*rfa*), and a deletion that covers genes involved in the synthesis of the vitamin biotin and in the repair of ultraviolet (UV) induced DNA damage (*uvr*B). The *rfa* mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The *uvr*B mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to histidine independence by many mutagens that cause base pair substitutions. TA100 is derived from TA1535 by the introduction of the drug resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error prone DNA repair that leads to many more mutations for a given dose of most mutagens (McCann *et al.*, 1975). In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker for detecting the presence of the plasmid in the cell

(Mortelmans and Stocker, 1979). The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens such as ICR 191. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by adding the plasmid pKM101, which makes it more sensitive to some mutagenic agents (Maron and Ames, 1983; Mortelmans and Zeiger, 2000).

The *E. coli* WP2 (*uvr*A) strain carries a mutation at the tryptophan allele, which is an auxotrophic mutation reverted by base pair substitution. The strain is deficient in the repair of UV induced DNA damage (*uvr*A) (Bridges, 1972; Green and Muriel, 1976; Mortelmans and Riccio, 2000) and thus has enhanced sensitivity to some mutagenic agents.

The objective of this study was to evaluate the ability of five bio-based fuels to induce genetic damage as detected by the *Salmonella-E. coli* microsome assay (Ames test). The purpose of this study was to provide data relating to the test substance's health effects, environmental effects, or environmental fate testing regulated by the U.S. Environmental Protection Agency (EPA). This study, therefore, was conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP). Testing procedures will be consistent with the Office of Prevention, Pesticides and Toxic Substances (OPPTS), Health Effects Test Guidelines, 870.5100 (U.S. EPA, 1988). The protocol and amendments are presented in Appendix A.

3.0 MATERIALS AND METHODS

3.1 Indicator Organisms

Two indicator organisms were used in this study. *Salmonella typhimurium* LT2, strains TA1535, TA1537, TA98, and TA100, were obtained from Dr. Bruce Ames, University of California, Berkeley, CA. *Escherichia coli*, strain WP2 (*uvr*A), were obtained from National Collection of Industrial and Marine Bacteria (NCIMB), Aberdeen, Scotland.

3.2 Test Fuels

Test fuels were supplied by the sponsor, Air Force Research Laboratory, Wright-Patterson Air Force Base, OH, and were reported by the sponsor to have purity greater than 99 percent (Table 1). The substances were all clear colorless liquids requiring storage conditions of 19°–26°C. GLP–compliant characterization was not provided by the Sponsor.

Table 1. Test fuel names and lot numbers

Fuel	Lot #s
Amyris	POSF5630
R-8	POSF5469
R-8 from Algae (Syntroleum/ Sapphire)	POSF5804
Swedish Biofuel	POSF5668
Fischer Tropsch (FT) fuel S-8	POSF4734

3.3 Dose Level Selection

The test substance and dose formulations were handled with the use of eye protection, gloves, and a protective smock or laboratory coat. A range-finding experiment was conducted with the test substances to determine a suitable dose range for the mutagenicity experiments. It was performed with *Salmonella* tester strain TA100, in the presence and absence of a metabolic activation mixture containing five percent (volume/volume) Aroclor 1254-induced rat-liver S9, using three plates per dose level. The highest dose level used in the range-finding experiment was 5 µl/plate, the recommended maximum test concentration. Dose solutions for the range-finding experiment with all five test substances were achieved by preparing a 0.05 ml/ml (5 µl/plate, 100 µl dosing volume) stock solution and serially diluting to obtain doses of 0.156, 0.313, 0.625, 1.25, and 2.5 µl/plate.

For the mutagenicity experiments the test substance was assessed in two independent experiments using five tester strains in the presence and absence of metabolic activation, with three plates per dose level. Dose selection for the mutagenicity experiments was made to assess the potential dose-response relationship and contain at least three nontoxic dose levels. All compounds were initially tested with and without five percent (volume/volume) S9 in the

metabolic activation system. Amyris was tested at doses of 0.005, 0.01, 0.02, 0.039, 0.078, and 0.156 µl/plate and retested with strains TA1535 and TA98 in the absence of metabolic activation at 0.0013, 0.0025, 0.005, 0.01, 0.02, and 0.039 µl/plate. All other compounds (R-8, R-8 from algae, Fischer Tropsch fuel S-8, and Swedish Biofuel) were tested at doses of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate.

In the second experiment for mutagenicity, all compounds were tested with and without 10 percent (volume/volume) S9 in the metabolic activation system. Amyris was tested at doses of 0.0013, 0.0025, 0.005, 0.01, 0.02, 0.039, and 0.078 µl/plate for the *Salmonella* strains, and at 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate for the strain WP2(*uvr*A). All other compounds (R-8, R-8 from algae, S-8, and Swedish Biofuel) were tested at dose levels 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate.

Test fuels were dissolved in dimethyl sulfoxide (DMSO, CAS No. 67-68-5). The solvent was purchased from Mallinckrodt Chemicals (Phillipsburg, NJ, lot numbers E32H05 (range-finding experiment) and H41J06 (mutagenicity experiments). DMSO is a clear, colorless liquid that was stored at 18°–25°C (E32H05) or 20°–24°C (H41J06). Characterization of the solvent was obtained from the manufacturer's Certificate of Analysis (CofA), which is included in Appendix B.

An aliquot of each test substance was added to DMSO to make a 0.05 ml/ml stock solution for the range-finding experiment. For the first mutagenicity experiment, a stock solution of 0.05 ml/ml was prepared for all test substances except Amyris, which was made at 0.00625 ml/ml. All test substances for the second mutagenicity were prepared at a stock concentration of 0.05 ml/ml. Each stock concentration was mixed on a vortex mixer for three times 30 seconds. In each of the experiments, serial dilutions were made from the initial stock solution and vortexed for 30 seconds between dilutions. Dose formulations were prepared at room temperature, under yellow light, and used on the day they were prepared. Unused dose formulations, not reserved for dose concentration analysis, were discarded immediately after use in the test system.

3.4 Positive Controls

Positive control substances are genotoxic in specific *Salmonella* strains. The positive control chemicals that are genotoxic without activation and the strains on which they are used are listed in Table 2. The positive control chemical that required activation is indicated in Table

3. Characterization of each positive control article was obtained from the manufacturer's CofA, which is included in Appendix B.

Positive controls also were dissolved DMSO from the same supplier. The lot numbers differed slightly from the DMSO used to dilute the test fuels; lot number E32H08 was used for the range-finding experiment and lot number H41J06 was used in the mutagenicity experiments.

Table 2. Positive control substances without activation

	Sodium azide	**9 Aminoacridine hydrochloride hydrate**	**2-Nitrofluorene**	**4-Nitroquinoline N-oxide**
Strain(s)	TA1535, TA100	TA1537	TA98	WP2 (*uvr*A)
CAS No.	26628-22-8	52417-22-8	607-57-8	56-57-5
Manufacturer	Sigma-Aldrich Corp. (St. Louis, MO)			
Lot No.	098K0052	07620TD	S43858	039K1332
Physical Description	White powder	Yellow powder	Dull yellow powder	Yellow powder
Storage Conditions	19°–24°C	19°–24°C	19°–24°C	–21° to –17°C
Dose/Plate	5 µg/50 µl	50 µg/50 µl	5 µg/50 µl	2.5 µg/50 µl

Table 3. Positive control substance with activation

Name	2-Anthramine (2-Aminoanthracene)		
CAS No.	613-13-8		
Manufacturer	Sigma-Aldrich Corp. (St. Louis, MO)		
Lot No.	12317CE		
Physical Description	Green gold powder		
Storage Conditions	19°–24°C		
Strain(s)	TA98, TA100	TA1535, TA1537	WP2 (*uvr*A)
Dose/Plate	2 µg/50 µl	4 µg/50 µl	20 µg/50 µl

3.5 Cytotoxicity Assessments

The indicator strains were kept frozen at -80ºC in nutrient broth supplemented with 10 percent sterile glycerol. New frozen stock cultures were made from single colony isolates. Cultures were inoculated into 50 ml Oxoid Nutrient Broth No. 2 (CM 67) and allowed to sit unshaken for 2 to 4 hours, then gently shaken (125 rpm) for 12 hours at 37ºC.

The metabolic activator, Aroclor 1254-induced rat liver homogenate preparation (S9), was purchased from Molecular Toxicology, Inc., Boone, NC. Lot No. 2447 (37.3 mg/ml protein) was used for the range-finding and the 1st mutagenicity experiments, while Lot No. 2565 (33.9 mg/ml protein) was used for the 2nd mutagenicity experiment. Liver enzymes are induced by injecting adult male Sprague Dawley rats with Aroclor 1254 (500 mg/kg) 5 days before they are sacrificed. The S9 consists of 9000 × g supernatant of liver homogenized in KCl (1 g wet weight of liver to 3 ml of 0.154M KCl). For quality control purposes, dilutions from each lot of S9, ranging from 0.2 to 10 percent in S9 mix, were tested for their ability to activate benzo(a)pyrene and 2-aminoanthracene to intermediates mutagenic to TA100 prior to product release. The metabolic activation mixture (Ames *et al.*, 1975; Maron and Ames, 1983) for the experiment(s) consisted of the components and amounts shown in Table 4.

Table 4. Preparation of metabolic activation mixture for 50 ml

Ingredient	5% S9 Mix (ml)	10% S9 Mix (ml)
Rat liver S9 (Aroclor 1254-induced)	2.5	5.0
$MgCl_2$ (0.4 M) and KCl (1.65 M) salts	1.0	1.0
Glucose-6-phosphate (1 M)	0.25	0.25
NADP (0.1 M)	2.0	2.0
Sodium phosphate buffer (0.2 M, pH 7.4)	25.0	25.0
Sterile distilled water	19.25	16.75

Plates were labeled with indelible ink to identify the test substance, the strain, the dose level, and the presence or absence of the metabolic activation system. The following ingredients were added to a sterile 13 × 100 mm test tube: (1) 2 ml of molten top agar; (2) 0.1 ml of indicator organisms (about 10^8 bacteria); (3) appropriate amount of the test substance, and (4) 0.5 ml of metabolic activation mixture or buffer. The test tube was placed in a 43°C

heating block. This mixture was stirred gently and then poured onto plates containing about 25 ml of minimal glucose agar (for WP2 (*uvr*A), the plates were supplemented with a trace of Oxoid nutrient broth). After the top agar had set, the plates were incubated at approximately 37°C for about 48 hours. The revertant colonies were counted after the incubation period; however, if the plates could not be immediately evaluated, they were refrigerated at approximately 4°C for one day until they could be counted.

Concurrent sterility, solvent, and positive controls were performed with each experiment. Sterility controls included separately plating out each test substance, metabolic activation mixture, and buffer. Solvent controls were performed for the positive controls and consisted of top agar, bacteria, metabolic activation mixture or buffer, and 50 µl DMSO, the solvent used to dissolve the positive control substances. The solvent control for the test substance, referred to as the zero dose, consisted of top agar, bacteria, metabolic activation mixture or buffer, and the solvent/diluent for the test substance. Positive controls were performed with each strain and consisted of top agar, bacteria, metabolic activation mixture or buffer, and 50 µl of the positive control substance.

The strains are analyzed for their genetic markers and for the presence of the plasmid whenever experiments are performed. The test plates were compared with the control plates for their revertant count and for the condition of the background bacterial lawn. Toxicity was estimated by several parameters: a substantial decrease in the number of revertant colonies on the test plates, clearing or absence of the background bacterial lawn growth, or formation of pinpoint nonrevertant colonies.

The revertant colonies were counted using an automated colony counter, when possible, to control bias. When accurate counts could not be obtained (e.g., because of precipitation on the plates), the colonies were counted manually using an electric probe colony counter. Data were collected using the Sorcerer Image Analysis System (version 2.2), and the Ames Study Manager (version 1.21), made by Perceptive Instruments (Suffolk, England). Counts from the automated colony counter were compared to manual counts prior to collecting data. A complete system calibration is performed annually.

3.6 Evaluation of Data

An experiment is considered valid when solvent controls are within ± 10 percent of historical limits for spontaneous revertants, when positive control mutagens elicit a positive

response (≥ 5-fold increase over the mean value for the solvent for the respective strain), and when there are at least three nontoxic dose levels (mutagenicity experiments). When experimental plates and sterility control plates indicate gross contamination, the results are not considered valid and the experiment is repeated. In addition, whenever experiments are performed, the strains are analyzed to confirm their genetic markers and the presence of the plasmid. If anomalies exist, the experiment is repeated.

The following criteria were used as guidelines for the interpretation of the data; however, the conclusions of the study were based upon the Study Director's evaluation and interpretation of the data. A test substance is considered a mutagen when a reproducible and statistically significant ($p < 0.01$) increase is observed at one or more dose levels. A statistically significant ($p < 0.01$) dose-related increase in the number of revertants is also considered a positive response. A test substance is considered a nonmutagen when the values for the dose levels are not reproducible or significant or when there is no statistically significant dose-related increase in the number of revertants. When a test substance cannot be identified clearly as a mutagen or nonmutagen, the results are classified as inconclusive.

3.7 Statistical Methods

The following statistical methods were used to evaluate the data. (1) Means and standard deviation were calculated from the individual plate counts; (2) Levene's test (Levene, 1960) was performed to determine if a significant difference exists among treatment variances; (3) treatments were compared with controls by using a one-tailed Dunnett's *t*-test (Dunnett, 1980) and within-levels pooled variance; and (4) evaluation of dose-relatedness for all treatments was made by regression analysis (Draper and Smith, 1981) of revertant counts versus the log of the concentrations (to allow inclusion of the zero dose, 1 was added to the dose before calculating the log). The significance of the regression was tested using a *t*-statistic. The statistical analyses were performed using the SAS analysis system: the data read into the SAS program version 9.1 and then the statistical analysis was run by version 6.12, using an Intel Centrino computer.

3.8 Regulatory Compliance

This study was conducted in compliance with 40 CFR Part 792, GLP standards, with the exceptions that the characterization of the test substances (identity, purity, and stability) and the supporting analytical chemistry of the dose formulations were not provided to the testing facility as this testing was not performed by the Sponsor nor the testing facility.

The protocol was amended on 12 March 2010 (Amendment No. 1) to specify the dose levels for the first experiment for mutagenicity, on 22 March 2010 (Amendment No. 2) to establish at least three nontoxic dose levels for Amyris with strains TA1535 and TA98 without metabolic activation, and on 29 March 2010 (Amendment No. 3) to specify the dose levels and S9 concentration to be used in the second experiment for mutagenicity.

All raw data, the original protocol and final report, relevant documents, and records specific to this study are the property of the Sponsor and will be stored at SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025. All records will be maintained for at least 10 years. At the end of the retention period, the Sponsor will be contacted regarding further disposition of these records. Wet specimens (e.g., colonies in agar) and samples of the control articles are not required to be retained.

4.0 RESULTS AND DISCUSSION

The presence of the appropriate genetic characteristics was verified for the strains used in this study. The results of the controls were acceptable for all experiments (see historical values in Appendix C) as well as the results of the sterility controls (metabolic activation mix, buffer, and a dilution of the test substance). There were an adequate number of nontoxic dose levels in the mutagenicity experiments to evaluate the test substance. Therefore, the criteria for a valid assay were met.

4.1 Range Finding Experiment

The range-finding experiment was performed with the five test substances using strain TA100 at doses representing 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate (100 µl dosing volume) in the presence and absence of a metabolic activation system (MA) containing 5

percent S9. The dose formulations at 5 µl/plate for all the test substances appeared to be slightly hazy when prepared. When the 5 µl/plate formulation of R-8 from algae was added to the test system, a slight precipitate was seen in the tubes and following the two-day incubation period, oil-like droplets were seen on the plates in the presence and absence of MA.

No precipitate was seen on the plates with the other test substances. No dose-related increase in the number of revertant colonies was seen with any of the test substances (see Appendix D).

Cytotoxicity, evident by a decrease in revertant counts, thinning of the background lawn, or the complete absence of revertant counts, was seen with Amyris at all doses in the presence and absence of MA, and with Swedish Biofuel at 5 µl/plate in the presence and absence of MA. All other compounds (R-8, R-8 from algae, and S-8) exhibited no signs of cytotoxicity.

4.2 Mutagenicity Experiments

The first experiment for mutagenicity was conducted with all five tester strains in the presence and absence of MA containing 5 percent S9. R-8, R-8 from algae, S-8, and Swedish Biofuel were tested at doses representing 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate. Due to the cytotoxicity observed in the range finding experiment with Amyris, doses were lowered to 0.005, 0.01, 0.02, 0.039, 0.078, and 0.156 µl/plate. The dose formulations at 5 µl/plate appeared to be slightly hazy/cloudy when prepared. When the 5 µl/plate formulation of R-8 from algae was added to the test system, a slight precipitate was seen in the tubes and following the two-day incubation period, oil-like droplets were seen on the plates in the presence and absence of MA. Amyris dose formulations were clear and colorless at the highest concentration. When Amyris was initially tested with strains TA1535 and TA98 in the absence of MA, only two dose levels (0.005 and 0.01 µl/plate) did not exhibit cytotoxicity (data not shown); therefore, this portion of the experiment was considered invalid and retested at 0.0013, 0.0025, 0.005, 0.01, 0.02, and 0.039 µl/plate in an effort to establish at least three nontoxic dose levels. The statistical analyses for the experiment are presented in Tables 5 through 9. Individual and mean plate counts are presented in Appendix E (Amyris, R-8, and Swedish Biofuel) and Appendix F (R-8 from algae and S-8).

No statistically significant increase in the number of revertant colonies was observed with any of the test substances, except for a slight increase seen with S-8 and TA1535 in the presence of MA which was statistically significant ($p < 0.01$) by regression analysis. Because

this increase was so slight, at its highest point <2-fold, and within the historical range for the strain, it was not considered to be a mutagenic response or biologically relevant. Cytotoxicity was evident by decreased revertant colony counts, thinning of the background lawn, and/or the appearance of pinpoint nonrevertant colonies. Cytotoxicity was seen with Amyris at doses ≥0.02 µl/plate (TA1535, -MA; TA98, -MA), ≥ 0.039 µl/plate (TA1535, +MA; TA1537, -/+MA; TA100, -/+MA), and ≥0.078 µl/plate (TA98, +MA). Cytotoxicity was seen with Swedish Biofuel at ≥2.5 µl/plate (TA1537, +MA) and at 5 µl/plate (TA1535, -/+MA; TA1537, -MA; TA98, -/+MA; TA100, -/+MA; WP2 (*uvr*A) -/+MA). No other signs of cytotoxicity were observed with the remaining test substances.

Table 5. Statistical analysis of the first mutagenicity experiment with Amyris

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535[a]	TA1537	TA98[a]	TA100	WP2*uvr*A
Amyris	0	0 µl	9 ± 3	11 ± 3	18 ± 4	110 ± 8	34 ± 6
	0	0.0013 µl	11 ± 5	NT	18 ± 6	NT	NT
	0	0.0025 µl	12 ± 6	NT	21 ± 5	NT	NT
	0	0.005 µl	12 ± 2	9 ± 3	18 ± 7	106 ± 14	26 ± 4
	0	0.010 µl	12 ± 3	8 ± 3	13 ± 2	112 ± 13	29 ± 4
	0	0.020 µl	Toxic†	6 ± 3	Toxic	97 ± 3	26 ± 4
	0	0.039 µl	Toxic	Toxic	Toxic	Toxic	31 ± 9
	0	0.078 µl	NT	Toxic	NT	Toxic	24 ± 3
	0	0.156 µl	NT	Toxic	NT	Toxic	27 ± 6
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			434.478 (571.09)	-478.95 (248.45)	-1268.3 (771.15)	-1291.3 (930.75)	-60.281 (55.202)
Y intercept (Standard error)			10.493 (1.273)	10.140 (1.226)	19.664 (1.720)	111.204 (4.591)	29.334 (1.533)

[a] = Experiment was performed with TA1535 and TA98 on 23 March 2010.
Toxic† = See Table 4 for individual plate evaluation
N = Not significant
NT = Not tested

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
Amyris	5	0 µl	13 ± 6	10 ± 2	36 ± 9	128 ± 10	43 ± 3
	5	0.005 µl	10 ± 5	8 ± 4	31 ± 5	132 ± 26	29 ± 9
	5	0.010 µl	10 ± 6	6 ± 2	32 ± 5	132 ± 17	30 ± 12
	5	0.020 µl	6 ± 3	8 ± 1	29 ± 4	118 ± 7	33 ± 2
	5	0.039 µl	Toxic†	Toxic	22 ± 6	Toxic	32 ± 7
	5	0.078 µl	Toxic	Toxic	Toxic	Toxic	33 ± 4
	5	0.156 µl	Toxic	Toxic	Toxic	Toxic	36 ± 3
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-704.61 (402.09)	-297.99 (218.98)	-755.03 (234.63)	-1343.4 (1413.8)	24.136 (74.604)
Y intercept (Standard error)			12.408 (1.983)	9.207 (1.080)	34.854 (2.028)	132.401 (6.974)	33.228 (2.072)

Toxic† = See Table 4 for individual plate evaluation
N = Not significant

Table 6. Statistical analysis of the first mutagenicity experiment with R-8

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8	0	0 µl	12 ± 5	11 ± 3	17 ± 3	110 ± 8	34 ± 6
	0	0.156 µl	9 ± 5	9 ± 4	24 ± 5	112 ± 10	33 ± 12
	0	0.313 µl	14 ± 2	7 ± 4	21 ± 7	109 ± 4	29 ± 4
	0	0.625 µl	16 ± 11	9 ± 4	21 ± 4	101 ± 13	31 ± 11
	0	1.25 µl	15 ± 5	7 ± 0	19 ± 3	108 ± 3	26 ± 6
	0	2.5 µl	15 ± 6	5 ± 2	18 ± 2	110 ± 14	35 ± 6
	0	5 µl	15 ± 1	6 ± 2	16 ± 5	109 ± 15	28 ± 5
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			4.647 (4.500)	-5.255 (2.344)	-5.278 (3.659)	0.404 (8.049)	-3.623 (5.989)
Y intercept (Standard error)			12.295 (1.774)	9.361 (0.924)	20.982 (1.443)	108.262 (3.173)	31.831 (2.361)

N = Not significant

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8	5	0 µl	17 ± 4	10 ± 2	36 ± 9	128 ± 10	43 ± 3
	5	0.156 µl	9 ± 2	13 ± 5	27 ± 4	99 ± 3	31 ± 6
	5	0.313 µl	7 ± 2	9 ± 2	32 ± 5	111 ± 12	27 ± 3
	5	0.625 µl	12 ± 4	12 ± 4	32 ± 8	114 ± 6	29 ± 2
	5	1.25 µl	10 ± 4	11 ± 3	33 ± 3	100 ± 12	34 ± 7
	5	2.5 µl	11 ± 3	9 ± 3	30 ± 1	107 ± 13	37 ± 6
	5	5 µl	17 ± 4	10 ± 5	29 ± 4	101 ± 10	31 ± 5
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			4.670 (3.957)	-2.423 (2.910)	-3.503 (4.424)	-18.705 (10.120)	-1.381 (5.546)
Y intercept (Standard error)			10.526 (1.560)	11.287 (1.147)	32.368 (1.744)	114.284 (3.990)	33.551 (2.186)

N = Not significant

Table 7. Statistical analysis of the first mutagenicity experiment with R-8 from algae

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8 from algae	0	0 µl	15 ± 5	7 ± 4	32 ± 2	124 ± 10	34 ± 8
	0	0.156 µl	12 ± 6	6 ± 3	22 ± 4	113 ± 6	20 ± 4
	0	0.313 µl	15 ± 3	7 ± 2	25 ± 2	106 ± 14	26 ± 13
	0	0.625 µl	10 ± 2	7 ± 3	24 ± 7	112 ± 8	26 ± 6
	0	1.25 µl	17 ± 4	5 ± 1	24 ± 1	97 ± 4	27 ± 4
	0	2.5 µl	11 ± 2	11 ± 3	25 ± 9	98 ± 22	33 ± 6
	0	5 µl	11 ± 5	7 ± 2	18 ± 4	91 ± 9	32 ± 5
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-4.137 (3.402)	2.393 (2.383)	-10.016 (4.397)	-35.204 (9.435)	8.048 (6.350)
Y intercept (Standard error)			14.317 (1.341)	6.341 (0.940)	27.100 (1.734)	116.297 (3.720)	26.148 (2.503)

N = Not significant

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8 from algae	5	0 µl	12 ± 7	10 ± 4	37 ± 3	123 ± 22	33 ± 6
	5	0.156 µl	10 ± 6	12 ± 2	33 ± 2	116 ± 9	35 ± 6
	5	0.313 µl	11 ± 5	8 ± 2	35 ± 7	117 ± 10	33 ± 6
	5	0.625 µl	12 ± 5	12 ± 3	30 ± 3	114 ± 16	35 ± 5
	5	1.25 µl	10 ± 4	8 ± 6	34 ± 4	106 ± 15	31 ± 6
	5	2.5 µl	10 ± 2	10 ± 5	29 ± 3	115 ± 11	30 ± 6
	5	5 µl	16 ± 3	9 ± 2	36 ± 5	100 ± 8	36 ± 9
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			4.290 (3.619)	-2.236 (2.797)	-1.270 (3.879)	-21.613 (10.465)	-0.249 (4.806)
Y intercept (Standard error)			10.305 (1.427)	10.375 (1.103)	33.660 (1.529)	119.333 (4.126)	33.550 (1.895)

N = Not significant

Table 8. Statistical analysis of the first mutagenicity experiment with S-8

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
S-8	0	0 µl	15 ± 5	7 ± 4	32 ± 2	124 ± 10	34 ± 8
	0	0.156 µl	25 ± 6	8 ± 3	26 ± 5	120 ± 13	33 ± 6
	0	0.313 µl	17 ± 6	5 ± 3	24 ± 7	109 ± 13	32 ± 7
	0	0.625 µl	17 ± 7	7 ± 4	28 ± 5	114 ± 22	33 ± 12
	0	1.25 µl	24 ± 9	7 ± 3	34 ± 4	118 ± 16	39 ± 3
	0	2.5 µl	14 ± 6	8 ± 1	22 ± 7	120 ± 22	37 ± 8
	0	5 µl	14 ± 6	7 ± 4	30 ± 2	116 ± 15	39 ± 5
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-6.545 (5.609)	0.470 (2.175)	-0.266 (5.123)	-1.821 (12.264)	7.844 (5.495)
Y intercept (Standard error)			19.789 (2.211)	6.671 (0.858)	28.031 (2.020)	117.823 (4.835)	33.018 (2.166)

N = Not significant

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
S-8	5	0 µl	12 ± 7	10 ± 4	37 ± 3	123 ± 22	33 ± 6
	5	0.156 µl	11 ± 4	12 ± 4	34 ± 4	143 ± 4	33 ± 4
	5	0.313 µl	9 ± 3	14 ± 4	31 ± 5	126 ± 17	42 ± 4
	5	0.625 µl	12 ± 3	12 ± 3	35 ± 3	147 ± 11	34 ± 3
	5	1.25 µl	11 ± 5	10 ± 3	38 ± 7	128 ± 5	40 ± 5
	5	2.5 µl	14 ± 4	13 ± 2	34 ± 3	125 ± 6	40 ± 3
	5	5 µl	23 ± 12	10 ± 2	37 ± 4	118 ± 6	31 ± 9
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			S	N	N	N	N
Slope (Standard error)			13.765 (4.783)	-1.608 (2.637)	1.813 (3.482)	-17.793 (11.382)	-1.393 (5.202)
Y intercept (Standard error)			8.984 (1.886)	11.903 (1.040)	34.512 (1.373)	135.300 (4.487)	36.602 (1.979)

S = Significant ($p<0.01$) by specified analyses
N = Not significant

Table 9. Statistical analysis of the first mutagenicity experiment with Swedish Biofuel

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
Swedish Biofuel	0	0 µl	12 ± 5	11 ± 3	17 ± 3	110 ± 8	34 ± 6
	0	0.156 µl	14 ± 4	11 ± 1	25 ± 8	120 ± 15	27 ± 1
	0	0.313 µl	9 ± 6	8 ± 2	21 ± 5	117 ± 5	24 ± 4
	0	0.625 µl	11 ± 4	8 ± 4	19 ± 1	107 ± 5	27 ± 6
	0	1.25 µl	9 ± 4	5 ± 1	22 ± 6	106 ± 14	32 ± 5
	0	2.5 µl	9 ± 2	7 ± 4	17 ± 9	82 ± 7	28 ± 8
	0	5 µl	Toxic†	Toxic	Toxic	Toxic	Toxic
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-7.915 (5.008)	-9.048 (3.198)	-4.197 (7.462)	-57.332 (13.477)	-0.964 (7.446)
Y intercept (Standard error)			12.533 (1.420)	10.221 (0.907)	20.957 (2.116)	119.310 (3.822)	28.596 (2.111)

N = Not significant
Toxic† = See Table 4 for individual plate evaluation

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
Swedish Biofuel	5	0 µl	13 ± 6	10 ± 2	36 ± 9	128 ± 10	43 ± 3
	5	0.156 µl	13 ± 4	8 ± 3	29 ± 7	143 ± 10	38 ± 8
	5	0.313 µl	12 ± 3	6 ± 4	33 ± 1	132 ± 20	39 ± 5
	5	0.625 µl	10 ± 4	5 ± 3	32 ± 9	135 ± 10	37 ± 4
	5	1.25 µl	11 ± 9	8 ± 4	31 ± 3	120 ± 26	35 ± 9
	5	2.5 µl	4 ± 1	Toxic†	26 ± 10	119 ± 13	30 ± 4
	5	5 µl	Toxic	Toxic	Toxic	Toxic	Toxic
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-14.923 (6.074)	-5.589 (6.654)	-13.185 (8.295)	-31.809 (19.231)	-19.650 (6.598)
Y intercept (Standard error)			13.538 (1.722)	8.299 (1.285)	32.053 (2.352)	136.386 (5.453)	41.053 (1.871)

N = Not significant
Toxic† = See Table 4 for individual plate evaluation

In the second experiment for mutagenicity, the dose levels for R-8, R-8 from algae, S-8, and Swedish Biofuel were 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate. Based on the results of the first experiment, the dose levels for Amyris were expanded in order to assess potential

mutagenicity and attempt to reach a cytotoxic dose with WP2 (*uvr*A). Therefore, dose levels ranged from 0.0013, 0.0025, 0.005, 0.01, 0.02, 0.039 and 0.078 µl/plate for the *Salmonella* strains (TA1535, TA1537, TA98, and TA100) and increased to 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate for the strain WP2 (*uvr*A). All testing was performed in the presence and absence of MA containing 10 percent S9. The dose formulations at 5 µl/plate appeared to be slightly hazy/cloudy when prepared. The statistical analyses for the experiment are presented in Tables 10 through 14. Individual and mean plate counts are presented in Appendix G. No statistically significant increase in the number of revertant colonies was observed with any of the test substances, except for a slight increase seen with R-8 and TA1537 in the absence of MA which was considered statistically significant ($p < 0.01$). Because this increase was so slight, at its highest point <2-fold, within the historical range for the strain, and not reproducible, it was not considered to be a mutagenic response or biologically relevant. Cytotoxicity was evident by decreased revertant colony counts and/or thinning of the background lawn. Cytotoxicity was seen with Amyris at doses ≥0.02 µl/plate (TA1535, -MA; TA98,-MA), ≥0.039 µl/plate (TA1535, +MA; TA1537, -/+MA; TA98, +MA; TA100, -MA), ≥0.078 µl/plate (TA100, +MA), and 5 µl/plate (WP2 (*uvr*A), -MA). Cytotoxicity was seen with Swedish Biofuel at ≥2.5 µl/plate (TA1537, +MA) and at 5 µl/plate (TA1535, -/+MA; TA1537, -MA; TA98, -/+MA; TA100, -/+MA; WP2 (*uvr*A) -/+MA). No other signs of cytotoxicity were observed with the remaining test substances.

Table 10. Statistical analysis of the first mutagenicity experiment with Amyris

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
Amyris	0	0 µl	17 ± 2	14 ± 1	23 ± 2	137 ± 26	24 ± 8
	0	0.0013 µl	15 ± 5	11 ± 3	26 ± 7	159 ± 14	NT
	0	0.0025 µl	13 ± 5	12 ± 4	22 ± 4	155 ± 12	NT
	0	0.005 µl	14 ± 3	11 ± 1	16 ± 6	139 ± 15	NT
	0	0.010 µl	11 ± 4	7 ± 2	23 ± 2	142 ± 12	NT
	0	0.020 µl	Toxic†	7 ± 3	Toxic	101 ± 10	NT
	0	0.039 µl	Toxic	Toxic	Toxic	Toxic	NT
	0	0.078 µl	Toxic	Toxic	Toxic	Toxic	28 ± 4
	0	0.156 µl	NT	NT	NT	NT	32 ± 1
	0	0.313 µl	NT	NT	NT	NT	25 ± 2
	0	0.625 µl	NT	NT	NT	NT	26 ± 3
	0	1.25 µl	NT	NT	NT	NT	22 ± 3
	0	2.5 µl	NT	NT	NT	NT	24 ± 2
	0	5 µl	NT	NT	NT	NT	Toxic
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-1159.6 (597.96)	-749.66 (203.94)	-537.24 (896.59)	-5446.8 (1382.9)	-7.730 (5.250)
Y intercept (Standard error)			15.820 (1.333)	12.424 (0.828)	23.074 (1.999)	153.971 (5.613)	27.459 (1.380)

NT = Not tested
Toxic† = See Table 4 for individual plate evaluation
N = Not significant

Table 10 (continued). Statistical analysis of the first mutagenicity experiment with Amyris

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
Amyris	10	0 µl	18 ± 4	12 ± 1	31 ± 5	147 ± 8	40 ± 7
	10	0.0013 µl	11 ± 4	9 ± 4	25 ± 2	121 ± 6	NT
	10	0.0025 µl	13 ± 2	8 ± 2	33 ± 3	117 ± 13	NT
	10	0.005 µl	11 ± 1	9 ± 3	33 ± 9	111 ± 6	NT
	10	0.010 µl	12 ± 3	11 ± 2	28 ± 3	122 ± 9	NT
	10	0.020 µl	11 ± 2	13 ± 1	34 ± 4	137 ± 8	NT
	10	0.039 µl	Toxic†	Toxic	Toxic	133 ± 2	NT
	10	0.078 µl	Toxic	Toxic	Toxic	Toxic	40 ± 11
	10	0.156 µl	NT	NT	NT	NT	42 ± 0
	10	0.313 µl	NT	NT	NT	NT	32 ± 11
	10	0.625 µl	NT	NT	NT	NT	36 ± 5
	10	1.25 µl	NT	NT	NT	NT	30 ± 4
	10	2.5 µl	NT	NT	NT	NT	31 ± 6
	10	5 µl	NT	NT	NT	NT	21 ± 2
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-384.27 (268.19)	430.634 (186.91)	471.231 (417.03)	559.634 (535.38)	-22.495 (5.151)
Y intercept (Standard error)			13.850 (1.088)	9.243 (0.759)	29.352 (1.692)	124.238 (3.918)	39.819 (1.901)

NT = Not tested
Toxic† = See Table 4 for individual plate evaluation
N = Not significant

Table 11. Statistical analysis of the second mutagenicity experiment with R-8

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8	0	0 µl	17 ± 2	14 ± 1	23 ± 2	137 ± 26	24 ± 8
	0	0.156 µl	16 ± 5	12 ± 4	21 ± 6	114 ± 6	24 ± 5
	0	0.313 µl	15 ± 3	12 ± 3	30 ± 4	107 ± 15	32 ± 3
	0	0.625 µl	19 ± 8	12 ± 1	25 ± 4	107 ± 5	26 ± 7
	0	1.25 µl	15 ± 1	16 ± 3	21 ± 4	110 ± 8	33 ± 6
	0	2.5 µl	14 ± 2	15 ± 4	23 ± 3	115 ± 18	21 ± 7
	0	5 µl	20 ± 3	17 ± 2	27 ± 9	97 ± 16	24 ± 7
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	S	N	N	N
Slope (Standard error)			1.747 (3.356)	5.488 (2.156)	1.229 (4.346)	-27.011 (13.426)	-3.444 (5.735)
Y intercept (Standard error)			16.008 (1.323)	12.475 (0.850)	23.970 (1.713)	120.355 (5.293)	27.398 (2.261)

S = Significant ($p<0.01$) by specified analyses
N = Not significant

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8	10	0 µl	18 ± 4	12 ± 1	31 ± 5	147 ± 8	40 ± 7
	10	0.156 µl	19 ± 8	16 ± 2	31 ± 4	157 ± 10	31 ± 3
	10	0.313 µl	16 ± 4	16 ± 3	35 ± 6	152 ± 8	34 ± 3
	10	0.625 µl	19 ± 3	14 ± 2	29 ± 8	144 ± 25	32 ± 8
	10	1.25 µl	12 ± 6	10 ± 3	30 ± 7	131 ± 10	36 ± 7
	10	2.5 µl	12 ± 2	13 ± 3	37 ± 1	130 ± 13	31 ± 8
	10	5 µl	19 ± 7	15 ± 3	33 ± 1	130 ± 21	36 ± 9
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-3.218 (4.345)	-0.827 (2.556)	3.463 (4.209)	-34.435 (11.544)	-0.683 (5.426)
Y intercept (Standard error)			17.474 (1.713)	13.863 (1.008)	31.216 (1.659)	151.832 (4.551)	34.535 (2.139)

N = Not significant

Table 12. Statistical analysis of the first mutagenicity experiment with R-8 from algae

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8 from algae	0	0 µl	17 ± 2	14 ± 1	23 ± 2	137 ± 26	24 ± 8
	0	0.156 µl	15 ± 1	12 ± 2	21 ± 2	111 ± 9	33 ± 7
	0	0.313 µl	15 ± 6	14 ± 5	22 ± 6	109 ± 3	22 ± 4
	0	0.625 µl	12 ± 8	12 ± 4	25 ± 9	110 ± 16	28 ± 2
	0	1.25 µl	13 ± 5	11 ± 1	15 ± 3	103 ± 13	26 ± 4
	0	2.5 µl	8 ± 0	5 ± 2	18 ± 1	96 ± 7	32 ± 6
	0	5 µl	10 ± 5	7 ± 3	22 ± 6	99 ± 2	29 ± 7
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-9.592 (3.497)	-10.751 (2.549)	-3.798 (4.268)	-35.838 (12.011)	5.129 (5.014)
Y intercept (Standard error)			15.451 (1.378)	13.841 (1.005)	22.169 (1.683)	119.770 (4.735)	26.152 (1.977)

N = Not significant

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
R-8 from algae	10	0 µl	18 ± 4	12 ± 1	31 ± 5	147 ± 8	40 ± 7
	10	0.156 µl	14 ± 2	10 ± 5	30 ± 1	126 ± 7	38 ± 2
	10	0.313 µl	12 ± 4	16 ± 2	26 ± 4	139 ± 9	30 ± 4
	10	0.625 µl	13 ± 2	16 ± 1	30 ± 5	135 ± 29	39 ± 3
	10	1.25 µl	10 ± 6	14 ± 4	34 ± 5	127 ± 19	34 ± 8
	10	2.5 µl	14 ± 2	13 ± 1	27 ± 5	146 ± 11	38 ± 10
	10	5 µl	12 ± 2	14 ± 2	31 ± 8	119 ± 8	39 ± 5
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-4.146 (2.935)	1.312 (2.632)	0.951 (4.171)	-15.983 (13.241)	2.573 (5.168)
Y intercept (Standard error)			14.414 (1.157)	13.184 (1.038)	29.529 (1.644)	138.861 (5.220)	36.193 (2.037)

N = Not significant

Table 13. Statistical analysis of the first mutagenicity experiment with S-8

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
S-8	0	0 µl	9 ± 3	14 ± 1	23 ± 2	137 ± 26	24 ± 8
	0	0.156 µl	16 ± 2	16 ± 3	25 ± 5	116 ± 18	28 ± 8
	0	0.313 µl	17 ± 7	12 ± 6	20 ± 5	125 ± 16	27 ± 6
	0	0.625 µl	13 ± 4	9 ± 2	19 ± 4	125 ± 13	31 ± 5
	0	1.25 µl	15 ± 2	12 ± 2	25 ± 6	121 ± 17	32 ± 4
	0	2.5 µl	15 ± 2	15 ± 4	20 ± 3	117 ± 17	26 ± 12
	0	5 µl	14 ± 3	10 ± 3	21 ± 4	98 ± 12	34 ± 3
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			1.827 (3.385)	-3.398 (2.989)	-2.731 (3.524)	-34.660 (13.751)	8.910 (5.471)
Y intercept (Standard error)			13.508 (1.335)	13.717 (1.155)	22.758 (1.389)	130.042 (5.421)	26.179 (2.157)

N = Not significant

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
S-8	10	0 µl	14 ± 4	12 ± 1	31 ± 5	147 ± 8	40 ± 7
	10	0.156 µl	15 ± 4	11 ± 3	34 ± 4	148 ± 7	36 ± 10
	10	0.313 µl	13 ± 5	13 ± 4	37 ± 4	160 ± 17	39 ± 9
	10	0.625 µl	12 ± 3	13 ± 2	36 ± 8	155 ± 6	32 ± 11
	10	1.25 µl	15 ± 6	14 ± 2	36 ± 10	153 ± 9	33 ± 4
	10	2.5 µl	13 ± 1	13 ± 1	33 ± 4	139 ± 23	30 ± 6
	10	5 µl	10 ± 3	16 ± 5	40 ± 2	144 ± 2	35 ± 6
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-3.308 (3.078)	5.230 (2.156)	6.285 (4.592)	-13.334 (10.102)	-7.169 (5.976)
Y intercept (Standard error)			14.072 (1.214)	11.694 (0.850)	33.526 (1.810)	153.555 (3.983)	37.259 (2.356)

N = Not significant

Table 14. Statistical analysis of the first mutagenicity experiment with Swedish Biofuel

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
Swedish Biofuel	0	0 µl	17 ± 2	14 ± 1	23 ± 2	137 ± 26	24 ± 8
	0	0.156 µl	15 ± 4	11 ± 5	24 ± 6	115 ± 16	28 ± 3
	0	0.313 µl	13 ± 5	7 ± 4	25 ± 2	109 ± 10	25 ± 8
	0	0.625 µl	18 ± 3	10 ± 2	23 ± 7	122 ± 20	28 ± 8
	0	1.25 µl	19 ± 3	10 ± 3	22 ± 8	155 ± 14	28 ± 6
	0	2.5 µl	13 ± 5	9 ± 2	22 ± 4	139 ± 17	27 ± 3
	0	5 µl	Toxic†	Toxic	Toxic	Toxic	Toxic
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-1.484 (5.086)	-4.902 (4.176)	-4.153 (5.895)	45.219 (26.496)	5.328 (7.202)
Y intercept (Standard error)			16.363 (1.442)	11.275 (1.184)	24.225 (1.672)	119.624 (7.513)	25.523 (2.042)

N = Not significant
Toxic† = See Table 6 for individual plate evaluation

Test Article	S9 (%)	Dose/ Plate	Mean ± Standard Deviation Revertants/Plate TA1535	TA1537	TA98	TA100	WP2*uvr*A
Swedish Biofuel	10	0 µl	18 ± 4	12 ± 1	31 ± 5	147 ± 8	40 ± 7
	10	0.156 µl	9 ± 4	10 ± 2	31 ± 4	147 ± 24	35 ± 9
	10	0.313 µl	15 ± 5	11 ± 5	33 ± 5	133 ± 4	31 ± 3
	10	0.625 µl	10 ± 2	11 ± 3	34 ± 2	123 ± 10	37 ± 5
	10	1.25 µl	11 ± 3	10 ± 2	36 ± 4	126 ± 10	35 ± 4
	10	2.5 µl	14 ± 2	Toxic†	28 ± 4	138 ± 9	33 ± 6
	10	5 µl	Toxic	Toxic	Toxic	Toxic	Toxic
ANALYSIS SUMMARY							
Levene's test			N	N	N	N	N
Dose response evaluated via log log regression			N	N	N	N	N
Slope (Standard error)			-3.981 (5.654)	-2.485 (5.126)	-2.917 (5.660)	-21.103 (17.739)	-6.209 (7.284)
Y intercept (Standard error)			13.577 (1.603)	11.103 (0.990)	32.737 (1.605)	140.253 (5.030)	36.333 (2.066)

N = Not significant
Toxic† = See Table 6 for individual plate evaluation

5.0 CONCLUSION

Amyris, R-8, R-8 from algae, Fischer Tropsch fuel S-8, and Swedish Biofuel were judged to be nonmutagenic under the test conditions used in this study; therefore, the test substances were determined to be negative in the bacterial reverse mutation assay.

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APPENDIX A. PROTOCOL AND AMENDMENTS

EVALUATION OF FIVE JET FUELS IN THE *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY

I. SRI STUDY NUMBER: G343-10

II. SPONSOR: Henry M. Jackson Foundation for the Advancement of Military Medicine
1401 Rockville Pike, Suite 600
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III. TESTING FACILITY: SRI International
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Proposed Experimental Start Date: 3 March 2010
Proposed Experimental Termination Date: 21 April 2010

IV. APPROVALS:

David R. Mattie — 1 Mar 10
David R. Mattie, PhD, DABT — Date
Sponsor's Authorized Representative

Edward S. Riccio — 3/2/2010
Edward S. Riccio, BS — Date
SRI Study Director

REVIEWED BY:

Thomas Bregante — 02 MAR 10
Thomas Bregante, BS — Date
SRI Quality Assurance Unit

V. OBJECTIVE AND PURPOSE OF STUDY

The objective of this study is to evaluate the ability of five bio-based jet fuels to induce genetic damage as detected by the *Salmonella-E. coli*/microsome assay.

The purpose of this study is to provide data relating to the test substance's health effects, environmental effects, or environmental fate testing regulated by the U.S. Environmental Protection Agency (EPA). This study, therefore, will be conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP). Testing procedures will be consistent with the Office of Prevention, Pesticides and Toxic Substances (OPPTS), Health Effects Test Guidelines, 870.5100.

VI. MATERIALS AND METHODS

A. Experimental Design

Route of Administration: Dissolved/diluted test article added to agar containing test system.

Reason for Route: Standard route to administer test article to test system for genotoxic evaluation of chemicals.

Design. A range-finding experiment will be conducted with the test substances to determine a suitable dose range for the mutagenicity experiments. The range-finding experiment will be performed with *Salmonella* tester strain TA100, in presence and absence of a rat-liver metabolic activation system (S9), using three plates per dose level, over a wide range of doses. For the mutagenicity experiments, the test substances will be assessed in two experiments using five tester strains in the presence and in the absence of metabolic activation, with three plates per dose level over at least five dose levels (to be added by protocol amendment). The test substances will initially be tested with 5% (v/v) S9 in the S9 mix. If no clear dose-related increase in the number of revertant colonies is observed with a test substance, a 10% (v/v) S9 mix will be used in the repeat experiment. If a mutagenic response is obtained with a test substance in the initial experiment with the 5% S9 mix, the assay will be repeated under the same conditions.

Justification of Dose Levels Selected. The highest dose level to be used in the range-finding experiment will be based on solubility or a dose representing 5 µl/plate. Dose selection for the mutagenicity experiments will be made to (1) assess a potential dose-response relationship, (2) include at least one dose that exhibits toxicity, or if a toxic level cannot be achieved, (3) contain a high dose of 5 µl/plate, the recommended maximum test concentration for a soluble noncytotoxic test substance.

Cytotoxicity Assessment. The test plates will be compared with the control plates for their revertant count and for the condition of the background bacterial lawn. Toxicity is estimated by several parameters: a substantial decrease in the number of revertant colonies on the test plates, clearing or absence of the background bacterial lawn growth, or formation of pinpoint nonrevertant colonies.

Endpoints Evaluated. The actual numbers of revertant colonies observed on the plates and the condition of the bacterial lawn growth.

B. Test and Control Substances

1. Test Substances:

Names / Lot Nos.:	1) R-8 / POSF5469 2) Amyris / POSF5630 3) Swedish Biofuel / POSF5668 4) R-8 from Algae (Syntroleum/Sapphire) / POSF5804 5) Fischer Tropsch (FT) fuel S-8 / POSF4734
Supplier:	Air Force Research Laboratory
Purity:	Reported by Sponsor to be greater than 99%
Physical Descriptions:	To be specified in the final report
Storage Conditions:	Store at room temperature, 15° to 30°C. Keep containers closed tightly. Use and store these materials in cool, dry, well-ventilated areas away from heat, direct sunlight, hot metal su*rfa*ces and all sources of ignition.
Characterization of Test Substances:	Characterization, identity, purity, and stability of the test substances will be the responsibility of the Sponsor and this information will not be contained in the final report.

Solvent

Name:	Dimethyl sulfoxide (DMSO)
CAS No.:	67-68-5
Manufacturer:	To be specified in the final report
Lot No.:	To be specified in the final report
Physical Description:	Clear, colorless liquid
Storage Conditions:	Room temperature, 15° to 30°C
Characterization of Solvent:	Characterization of the solvent will be obtained from the manufacturer's Certificate of Analysis.
Preparation of Dose	An aliquot of the test substance will be prepared in

Formulations:	the solvent at a maximum concentration of 0.1 ml/ml. If the test substance is not soluble, it will be gradually diluted until solubility is achieved. Once the stock concentration is prepared, serial dilution will be made from the initial stock. All dose formulations will be prepared at room temperature and mixed thoroughly on a mixing device (at least 5 seconds) to ensure homogeneity and adequate solubility. Unless otherwise specified, dose formulations not used on the day of preparation will be stored refrigerated and protected from light. They will be brought to room temperature prior to exposure to the test system.
Characterization of Dose Formulations:	Assays to verify the stability, homogeneity, and concentration of each test substance in the vehicle will be the responsibility of the Sponsor and will not be contained in the final report.
Disposition:	Unused bulk test substance will be returned to the Sponsor. Unused dose formulations, not reserved for dose concentration analysis, will be discarded immediately after use in the test system.
Test Substance Handling:	The test substances and dose formulations will be handled with the use of eye protection, gloves, and a protective smock or laboratory coat.

Positive Controls without Activation

For Strains TA1535 & TA100:	Sodium azide
CAS No.:	26628-22-8
Manufacturer:	To be specified in the final report
Lot No.:	To be specified in the final report
Physical Description:	To be specified in the final report
Storage Conditions:	Room temperature, 15° to 30°C
Dose/Plate:	5 µg/50 µl
For Strain TA1537:	9-Aminoacridine hydrochloride
CAS No.:	52417-22-8
Manufacturer:	To be specified in the final report
Lot No.:	To be specified in the final report
Physical Description:	To be specified in the final report
Storage Conditions:	Room temperature, 15° to 30°C
Dose/Plate:	50 µg/50 µl

For Strain TA98:	2-Nitrofluorene
CAS No.:	607-57-8
Manufacturer:	To be specified in the final report
Lot No.:	To be specified in the final report
Physical Description:	To be specified in the final report
Storage Conditions:	Room temperature, 15° to 30°C
Dose/Plate:	5 µg/50 µl

For Strain WP2 (*uvr*A):	4-Nitroquinoline N-oxide
CAS No.:	56-57-5
Manufacturer:	To be specified in the final report
Lot No.:	To be specified in the final report
Physical Description:	To be specified in the final report
Storage Conditions:	Frozen, −20° to −10°C
Dose/Plate:	2.5 µg/50 µl

Positive Control with Activation

Name:	2-Anthramine (2-Aminoanthracene)
CAS No.:	613-13-8
Manufacturer:	To be specified in the final report
Lot No.:	To be specified in the final report
Physical Description:	To be specified in the final report
Storage Conditions:	Room temperature, 15° to 30°C
Dose/Plate:	2 µg/50 µl (TA98, TA100), 4 µg/50 µl (TA1535, TA1537) & 20 µg/50 µl (WP2 (*uvr*A)) in the presence of activation
Characterization of Positive Controls:	Characterization of each positive control substance will be obtained from the manufacturer's Certificate of Analysis.

Solvent for the Positive Controls

Name:	Dimethyl sulfoxide (DMSO)
CAS No.:	67-68-5
Manufacturer:	To be specified in the final report
Lot No.:	To be specified in the final report
Physical Description:	Clear, colorless liquid
Storage Conditions:	Room temperature, 15° to 30°C

Characterization of Solvent:	Characterization of the solvent will be obtained from the manufacturer's Certificate of Analysis.

C. Test System

Test System Justification. Microbial mutagenicity assays are capable of rapidly detecting the mutagenic activity of many materials, including a wide range of chemical classes. Many chemicals that elicit a mutagenic response in the *Salmonella* assay have been shown to be potentially mutagenic and carcinogenic to humans and laboratory animals. One advantage of using the procedure with *E. coli* is that this strain has an A-T base-pair at the critical mutation site and thus is sensitive to some agents that are not detected by the *Salmonella* strains. Because microbial mutagenicity assays are short-term, sensitive, and reliable tests for assessing mutagenic potential, their use for genotoxic evaluation of chemicals is appropriate.

Indicator Organisms

Species:	*Salmonella typhimurium* LT2
Strains:	TA1535, TA1537, TA98, and TA100
Source:	Dr. Bruce Ames, University of California, Berkeley
Species:	*Escherichia coli*
Strain:	WP2 (*uvr*A)
Source:	National Collection of Industrial and Marine Bacteria (NCIMB), Aberdeen, Scotland

Description of the Strains. The *Salmonella* tester strains have mutations in the histidine operon, a mutation that leads to a defective lipopolysaccharide coat (*rfa*), and a deletion that covers genes involved in the synthesis of the vitamin biotin and in the repair of ultraviolet (UV)-induced DNA damage (*uvr*B). The *rfa* mutation makes the strains more permeable to many large molecules, thereby increasing the mutagenic effect of these molecules. The *uvr*B mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically damaged DNA and thereby enhances the strains' sensitivity to some mutagenic agents. Strain TA1535 is reverted to histidine independence by many mutagens that cause base-pair substitutions. TA100 is derived from TA1535 by the introduction of the drug resistance transfer factor, plasmid pKM101. This plasmid is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens (McCann *et al.*, 1975). In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker for detecting the presence of the plasmid in the cell (Mortelmans and Stocker, 1979). The presence of this plasmid also makes strain TA100 sensitive to some frameshift mutagens such as ICR-191. Strains TA1537 and TA1538 are reverted by many frameshift mutagens. Strain TA98 is derived from TA1538 by adding the plasmid pKM101, which makes it more sensitive to some mutagenic agents (Maron and Ames, 1983; Mortelmans and Zeiger, 2000).

The *E. coli* WP2 (*uvr*A) strain carries a mutation at the tryptophan allele, which is an auxotrophic mutation reverted by base-pair substitution. The strain is deficient in the repair of UV-induced DNA damage (*uvr*A) (Bridges, 1972; Green and Muriel, 1976; Mortelmans and Riccio, 2000) and thus has enhanced sensitivity to some mutagenic agents.

Test System Identification: The strains are analyzed for their genetic markers and for the presence of the plasmid whenever experiments are performed.

Culture Conditions: The indicator strains are kept frozen at -80°C in nutrient broth supplemented with 10% sterile glycerol. New frozen stock cultures are made from single colony isolates. Cultures are inoculated into 50 ml Oxoid Nutrient Broth No. 2 (CM 67) and allowed to sit unshaken for 2 to 4 hours, then gently shaken (100 to 125 rpm) for about 11 to 14 hours at 37°C.

Identification: Plates are labeled with indelible ink to identify the test substance, the strain, the dose level, and the presence or absence of the metabolic activation system.

Metabolic Activation

Supplier: Molecular Toxicology, Inc., Boone, NC

Description: Aroclor 1254-induced rat liver homogenate preparation (S9)

Preparation: Liver enzymes are induced by injecting adult male Sprague-Dawley rats with Aroclor 1254 (500 mg/kg) 5 days before they are sacrificed. The S9 consists of 9000 × *g* supernatant of liver homogenized in KCl (1 g wet weight of liver to 3 ml of 0.154M KCl).

Quality Control: Dilutions from each lot of S9, ranging from 0.2 to 10% in S9 mix, were tested for their ability to activate benzo(a)pyrene and 2-aminoanthracene to intermediates mutagenic to TA100 prior to product release.

Metabolic Activation The metabolic activation mixture (Ames

Mixture: *et al.*, 1975; Maron and Ames, 1983) for the experiment(s) will consist of the components and amounts shown below.

PREPARATION OF METABOLIC ACTIVATION MIXTURE FOR 50 ml

Ingredient	5% S9 Mix (ml)	10% S9 Mix (ml)
Rat liver S9 (Aroclor 1254-induced)	2.5	5.0
$MgCl_2$ (0.4 M) and KCl (1.65 M) salts	1.0	1.0
Glucose-6-phosphate (1 M)	0.25	0.25
NADP (0.1 M)	2.0	2.0
Sodium phosphate buffer (0.2 M, pH 7.4)	25.0	25.0
Sterile distilled water	19.25	16.75

D. Experimental Procedure

To a sterile 13 × 100-mm test tube placed in a 43°C heating block will be added:

(1) 2 ml of molten top agar

(2) 0.1 ml of indicator organisms (about 10^8 bacteria)

(3) appropriate amount of the test substance

(4) 0.5 ml of metabolic activation mixture or buffer.

This mixture will be stirred gently, and then poured onto plates containing about 25 ml of minimal glucose agar. After the top agar has set, the plates will be incubated at ~37°C for about 48 hours. The revertant colonies will be counted after the incubation period; however, if the plates cannot be immediately evaluated, they will be refrigerated at ~4°C until they can be counted.

Concurrent sterility, solvent, and positive controls will be performed with each experiment. Sterility controls will include separately plating out each test substance, metabolic activation mixture, and buffer. Solvent controls will be performed for the positive controls and will consist of top agar, bacteria, metabolic activation mixture or buffer, and 50 µl DMSO, the solvent used to dissolve the positive control substances. The solvent control for the test

substance, referred to as the zero dose, will consist of top agar, bacteria, metabolic activation mixture or buffer, and the solvent/diluent for the test substance. Positive controls will be performed with each strain and consist of top agar, bacteria, metabolic activation mixture or buffer, and 50 µl of the positive control substance.

E. Data Collection

Control of Bias. Bias is controlled by collecting data with an automated colony counter when possible.

Colony Counting. The revertant colonies will be counted using an automated colony counter, Sorcerer Image Analysis System (version 2.2) and the data managed through the Ames Study Manager (version 1.21), both manufactured by Perceptive Instruments (Suffolk, England). When accurate counts cannot be obtained (e.g., because of precipitation on the plates), the colonies will be counted manually using an electric probe colony counter.

F. Evaluation of Data

Criteria for Valid Assay. An experiment is considered valid when solvent controls are within ± 10% of historical limits for spontaneous revertants, when positive control mutagens elicit a positive response (≥ 5-fold increase over the mean value for the solvent for the respective strain), and when there are at least three nontoxic dose levels (mutagenicity experiments). When experimental plates and sterility control plates indicate gross contamination, the results are not considered valid and the experiment is repeated. In addition, whenever experiments are performed, the strains are analyzed to confirm their genetic markers and the presence of the plasmid. If anomalies exist, the experiment is repeated.

Statistical Methods. (1) Means and standard deviation will be calculated from the individual plate counts; (2) Levene's test (Levene, 1960) will be performed to determine if a significant difference exists among treatment variances; (3) treatments will be compared with controls by using a one-tailed Dunnett's *t*-test (Dunnett, 1980) and within-levels pooled variance; and (4) evaluation of dose-relatedness for all treatments will be made by regression analysis (Draper and Smith, 1981) of revertant counts versus the log of the concentrations (to allow inclusion of the zero dose, 1 will be added to the dose before calculating the log). The significance of the regression will be tested using a *t*-statistic.

Criteria for Interpretation. The following criteria will be used as guidelines for the interpretation of the data; however, the conclusions of the study will be based upon the Study Director's evaluation and interpretation of the data.

Positive. A test substance will be considered a mutagen when a reproducible and statistically significant ($p < 0.01$) increase in revertants is observed at one or more dose levels. A statistically significant ($p < 0.01$) dose-related increase in the number of revertants will also be considered a positive response.

Negative. A test substance will be considered a nonmutagen when the values for the dose levels are not reproducible or significant or when there is no statistically significant dose-related increase in the number of revertants.

Inconclusive. When a test substance cannot be identified clearly as a mutagen or nonmutagen, the results will be classified as inconclusive.

VII. REGULATORY COMPLIANCE

A. Good Laboratory Practice (GLP) Compliance

This study will be conducted in compliance with 40 CFR Part 792, Good Laboratory Practice Standards (GLP), with the exceptions that the characterization of the test substances (identity, purity, and stability) and the supporting analytical chemistry of the dose formulations will not be provided to the testing facility as this testing will not be performed by the Sponsor nor the testing facility.

B. Standard Operating Procedures

All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the Standard Operating Procedures of the laboratory, and any deviations will be documented.

C. Protocol Amendments

All changes in or revisions of an approved protocol and the reasons for them will be documented and signed and dated by the Study Director and the Sponsor's Representative. Amendments will be maintained with the protocol. Verbal approval for changes in the protocol may be granted by the Sponsor's Representative, but a written amendment will follow.

D. Retention of Records and Study Samples

All raw data, the original protocol and final report, relevant documents, and records specific to this study are the property of the Sponsor and will be stored at SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025. All records will be maintained for at least 10 years. At the end of the retention period, the Sponsor will be contacted regarding further disposition of these records. Wet

specimens (e.g., colonies in agar) and samples of the control substances are not required to be retained.

VIII. REPORTING

The final report will describe the study design, procedures, and findings and will present an analysis and summary of the data followed by the conclusions derived from the analyses. A draft report will be issued prior to submission of the final report.

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Mortelmans, K., E. Zeiger. The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res. **455**: 29-60, 2000.

Protocol Amendment No. 1

PROTOCOL TITLE:	EVALUATION OF FIVE JET FUELS IN THE *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY
SRI Study Number:	G343-10
Sponsor:	Henry M. Jackson Foundation for the Advancement of Military Medicine
Sponsor's Representative:	David R. Mattie, PhD, DABT

This amendment modifies the following sections of the study protocol:

VI. MATERIALS AND METHODS, A. Experimental Design, page 2 of 12

Add to the protocol, the following dose levels to be used for the first experiment for mutagenicity.

Based on information derived from the range-finding experiment, the first experiment for mutagenicity with R-8, Swedish Biofuel, R-8 from Algae, and Fischer Tropsch fuel S-8 will be conducted at doses of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate. Amyris will be conducted at doses of 0.005, 0.010, 0.020, 0.039, 0.078, and 0.156 µl/plate. All testing will be performed in the presence and absence of metabolic activation containing 5% S9.

Reason: This addition to the protocol is necessary to establish the dose levels for the first mutagenicity experiment based on the results of the range-finding experiment.

Impact on the study: These dose levels should allow for the assessment of potential mutagenicity and contain at least three nontoxic dose levels.

APPROVALS

David R. Mattie

David R. Mattie, PhD, DABT
Sponsor's Representative

10 Mar 10
Date

Edward S. Riccio

Edward S. Riccio
Study Director

3/12/2010
Date

Protocol Amendment No. 2

PROTOCOL TITLE:	EVALUATION OF FIVE JET FUELS IN THE *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY
SRI Study Number:	G343-10
Sponsor:	Henry M. Jackson Foundation for the Advancement of Military Medicine
Sponsor's Representative:	David R. Mattie, PhD, DABT

This amendment modifies the following sections of the study protocol:

VI. MATERIALS AND METHODS, A. Experimental Design, page 2 of 12

Add to the protocol, the following dose levels to be used for the first experiment for mutagenicity of Amyris with strains TA1535 and TA98 in the absence of metabolic activation.

Based on information derived from the first experiment for mutagenicity with Amyris, doses for TA1535 and TA98 in the absence of metabolic activation will be 0.0013, 0.0025, 0.005, 0.010, 0.020, and 0.039 µl/plate.

Reason: This addition to the protocol is necessary to establish at least three nontoxic dose levels for the first mutagenicity experiment under the selected test conditions. Excessive cytotoxicity was observed with TA1535 and TA98 in the absence of metabolic activation in the first mutagenicity experiment.

Impact on the study: These dose levels should allow for the assessment of potential mutagenicity and contain at least three nontoxic dose levels.

APPROVALS

David R. Mattie — 19 Mar 10

David R. Mattie, PhD, DABT
Sponsor's Representative — Date

Edward S. Riccio — 3/22/2010

Edward S. Riccio
Study Director — Date

Protocol Amendment No. 3

PROTOCOL TITLE:	EVALUATION OF FIVE JET FUELS IN THE *SALMONELLA-ESCHERICHIA COLI*/MICROSOME PLATE INCORPORATION ASSAY
SRI Study Number:	G343-10
Sponsor:	Henry M. Jackson Foundation for the Advancement of Military Medicine
Sponsor's Representative:	David R. Mattie, PhD, DABT

This amendment modifies the following sections of the study protocol:

VI. MATERIALS AND METHODS, A. Experimental Design, page 2 of 12

Add to the protocol, the following dose levels to be used for the second experiment for mutagenicity.

Based on information derived from the first experiment for mutagenicity, the second experiment for mutagenicity with R-8, Swedish Biofuel, R-8 from Algae, and Fischer Tropsch fuel S-8 will be conducted at doses of 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate. Amyris will be conducted at doses of 0.0013, 0.0025, 0.005, 0.010, 0.020, 0.039, and 0.078 µl/plate for *Salmonella* strains TA1535, TA1537, TA98, and TA100 and at doses of 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, and 5 µl/plate for *E. coli* strain WP2 (*uvr*A). All testing will be performed in the presence and absence of metabolic activation containing 10% S9.

Reason: This addition to the protocol is necessary to establish at least three nontoxic dose levels and to increase the level of metabolic activation to be used for the second mutagenicity experiment.

Impact on the study: These dose levels should allow for the assessment of potential mutagenicity and contain at least three nontoxic dose levels.

APPROVALS

David R. Mattie — 29 Mar 10

David R. Mattie, PhD, DABT
Sponsor's Representative — Date

Edward S. Riccio — 3/29/2010

Edward S. Riccio
Study Director — Date

APPENDIX B. CERTIFICATES OF ANALYSIS

Mallinckrodt CHEMICALS

Dimethyl Sulfoxide

AR® (ACS)

Product No. 4948
Lot No. E32H05
Release Date 08/09/2007

Certificate of Analysis

TEST	SPECIFICATION	RESULT
Meets A.C.S. Specifications		
Assay ($(CH_3)_2SO$) (by GC, corrected for water)	99.9 % min.	99.9 %
Appearance (clear, colorless liquid)	Passes Test	Passes Test
Residue after Evaporation	0.01 % max.	< 0.0001 %
Titrable Acid (meq/g)	0.001 max.	0.0002
Water (H_2O)(by coulometry)	0.1 % max.	0.02 %

For Laboratory, Research or Manufacturing Use

Country of Origin: USA

ISO

Phillipsburg, NJ 9001:2000 & 14001:1996
Paris, KY 9001:2000
Mexico City, Mexico 9001:2000
Deventer, Holland 9001:2000 & 14001:1996
Selangor, Malaysia 9001:2000

Mary M. Mallory

For questions on this Certificate of Analysis please contact Technical Services at 1-800-582-2537 or 908-859-2151
Mallinckrodt Baker, Inc. • 222 Red School Lane • Phillipsburg, NJ 08865 • Phone: 908.859.2151 • Fax: 908.859.6905

Mallinckrodt CHEMICALS

Dimethyl Sulfoxide

AR® (ACS)

Product No. 4948
Lot No. H41J06
Release Date 10/05/2009

Certificate of Analysis

TEST	SPECIFICATION	RESULT
Meets A.C.S. Specifications		
Assay ($(CH_3)_2SO$) (by GC, corrected for water)	99.9 % min.	99.9 %
Appearance (clear, colorless liquid)	Passes Test	Passes Test
Residue after Evaporation	0.01 % max.	< 0.001 %
Titrable Acid (meq/g)	0.001 max.	0.0002
Water (H_2O)(by coulometry)	0.1 % max.	0.06 %

For Laboratory, Research or Manufacturing Use

Country of Origin: USA

ISO

Phillipsburg, NJ 9001:2000 & 14001:2001
Paris, KY 9001:2000
Mexico City, Mexico 9001:2000
Deventer, Holland 9001:2000 & 14001:2001
Selangor, Malaysia 9001:2000

Mary M. Mallory

For questions on this Certificate of Analysis please contact Technical Services at 1-800-582-2537 or 908-859-2151
Mallinckrodt Baker, Inc. • 222 Red School Lane • Phillipsburg, NJ 08865 • Phone: 908.859.2151 • Fax: 908.859.6905

Certificate of Analysis

SIGMA-ALDRICH

Product Name	2-Nitrofluorene, 98%
Product Number	N16754
Product Brand	ALDRICH
CAS Number	607-57-8
Molecular Formula	$C_{13}H_9NO_2$
Molecular Weight	211.22

TEST	**LOT S43858 RESULTS**
QC Acceptance date	14-AUG-2007
APPEARANCE - COLOUR	YELLOW-TAN
APPEARANCE - STATE	POWDER
ELEMENTAL ANALYSIS - CARBON	74.0%
ELEMENTAL ANALYSIS - HYDROGEN	4.3%
ELEMENTAL ANALYSIS - NITROGEN	6.6%
HPLC - PURITY	97.9%
IR SPECTROSCOPY - FTIR SPECTRUM	CONFORMS TO STRUCTURE

Claudia Mayer, Manager
Quality Control
Steinheim Germany

Certificate of Analysis

SIGMA-ALDRICH

Product Name	9-Aminoacridine hydrochloride monohydrate, 98%
Product Number	A38401
Product Brand	ALDRICH
CAS Number	52417-22-8
Molecular Formula	$C_{13}H_{10}N_2 \cdot HCl \cdot H_2O$
Molecular Weight	248.71

TEST	SPECIFICATION	LOT 07620TD RESULTS
APPEARANCE	YELLOW OR YELLOW-GREEN POWDER	YELLOW POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.	CONFORMS TO STRUCTURE.
TITRATION	97.5% - 102.5% (WITH AGNO3)	98.7% (WITH SILVER NITRATE)
HIGH PRESSURE LIQUID CHROMATOGRAPHY	97.5% (MINIMUM)	99.9%
TITRATION	TYPICALLY 3%-8% H2O (WITH "KARL FISCHER RGT)	7.0% H2O (WITH "KARL FISCHER" REAGENT)
PRODUCT CROSS REFERENCE INFORMATION		REPLACES PRODUCT NUMBER A1135
QUALITY CONTROL ACCEPTANCE DATE		DECEMBER 2005

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Certificate of Analysis

SIGMA-ALDRICH

Product Name	2-Aminoanthracene, 96%
Product Number	A38800
Product Brand	ALDRICH
CAS Number	613-13-8
Molecular Formula	$C_{14}H_{11}N$
Molecular Weight	193.24

TEST	SPECIFICATION	LOT 12317CE RESULTS
APPEARANCE	GOLD TO TAN TO OLIVE GREEN POWDER	GREEN-GOLD POWDER
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD	CONFORMS TO STRUCTURE.
ELEMENTAL ANALYSIS	CARBON 83.1%-91.8%	CARBON 86.7%
	NITROGEN 6.9%- 7.6%	NITROGEN 7.0%
HIGH PRESSURE LIQUID CHROMATOGRAPHY	95.5% (MINIMUM)	99.9%
SOLUBILITY	50MG/ML(5%), DMF; CLEAR TO OPAQUE, YELLOW	5%, DMF; OPAQUE, DARK GREEN SOLUTION
PRODUCT CROSS REFERENCE INFORMATION		REPLACES PRODUCT NUMBER A1381
QUALITY CONTROL ACCEPTANCE DATE		MARCH 2006

Barbara Rejzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Certificate of Analysis

SIGMA-ALDRICH®

Product Name	Sodium azide, *ReagentPlus*®, ≥99.5%
Product Number	S2002
Product Brand	SIAL
CAS Number	26628-22-8
Molecular Formula	NaN_3
Molecular Weight	65.01

TEST	SPECIFICATION	LOT 098K0052 RESULTS
APPEARANCE	WHITE POWDER	CONFORMS
PURITY BY TITRATION	> OR = 99.5%	99.8% (SUPPLIER TEST RESULT)
RECOMMENDED RETEST	5 YEARS	SEPTEMBER 2013
QC RELEASE DATE		SEPTEMBER 2008
PRODUCT CROSS REFERENCE INFORMATION		REPLACEMENT FOR ALDRICH #199931

Rodney Burbach, Manager
Quality Control
St. Louis, Missouri USA

Certificate of Analysis

SIGMA-ALDRICH

Product Name	4-Nitroquinoline *N*-oxide
Product Number	N8141
Product Brand	ALDRICH
CAS Number	56-57-5
Molecular Formula	$C_9H_6N_2O_3$
Molecular Weight	190.16
Storage Temp	−20°C

TEST	SPECIFICATION	LOT 039K1332 RESULTS
Appearance (Color)	Yellow to Brown	Yellow
Appearance (Form)	Powder	Powder
Solubility (Turbidity)	Clear to Slightly Hazy	Clear
Solubility (Color)	Yellow to Orange	Yellow with an Orange Cast
	At 25 mg/ml in acetone	
Carbon	55.4 - 58.3 %	56.8 %
Nitrogen	14.4 - 15.1 %	14.7 %
Purity (HPLC)	≥98 %	99 %
Specification Date:		MAR 2009
Date of QC Release:		APR 2009
Print Date:		APR 21 2009

Rodney Burbach

Rodney Burbach, Manager
Quality Control
St. Louis, Missouri USA

APPENDIX C. HISTORICAL VALUES FOR SPONTANEOUS REVERTANTS AND POSITIVE CONTROLS

(Note: Historical data includes GLP studies conducted at SRI International from 1/05 to 3/10)

HISTORICAL VALUES
FOR SPONTANEOUS REVERTANTS AND POSITIVE CONTROLS

Strain	Spontaneous Revertants
TA1535	5 - 35
TA1537	1 - 20
TA98	10 - 45
TA100	90 - 210
WP2*uvr*A	10 - 50

Strain	Positive Control	S9 (%)	Dose/Plate	Range
TA1535	sodium azide	0	5 µg	780 - 2680
TA1537	9-aminoacridine	0	50 µg	108 - 800
TA98	2-nitrofluorene	0	5 µg	640 - 2791
TA100	sodium azide	0	5 µg	860 - 2630
WP2*uvr*A	4-Nitroquinoline-N-oxide	0	2.5 µg	1285 - 4511
TA1535	2-anthramine	5/10	4 µg	215-652/180-500
TA1537	2-anthramine	5/10	4 µg	265-920/225-710
TA98	2-anthramine	5/10	2 µg	865-3905/805-2790
TA100	2-anthramine	5/10	2 µg	1065-4800/1005-3085
WP2*uvr*A	2-anthramine	5/10	20 µg	225-920/140-775

APPENDIX D. INDIVIDUAL AND MEAN PLATE COUNTS: RANGE FINDING EXPERIMENT WITH FIVE JET FUELS

Table A-1. Amyris plate counts

Study Name: G343-10 (Amyris)
Experiment: Range Finder G343-10 (Amyris POSF5630)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Amyris)
Date Plated: 3/3/2010
Date Counted: 3/5/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Amyris (POSF5630)**	0.156 µl	*65.0*	*9.5*	*0.6*	55 H, 74 H, 66 H
		0.313 µl	*64.3*	*20.0*	*0.6*	57 I, 49 I, 87 I
		0.625 µl	*57.7*	*11.9*	*0.5*	63 I, 44 I, 66 I
		1.25 µl	*0.0*	*0.0*	*0.1*	0 I, 0 I, 0 I
		2.5 µl	*0.0*	*0.0*	*0.0*	0 I, 0 I, 0 I
		5 µl	*0.0*	*0.0*	*0.0*	0 I, 0 I, 0 I
	DMSO		*114.3*	*4.0*		118, 110, 115
	Untreated Control		*128.2*	*11.4*		134, 109, 130, 129, 139
TA100	**SA**	5 µg	*1510.7*	*33.4*	*13.2*	1495, 1549, 1488
TA100	**DMSO**	–	*105.7*	*12.7*	*0.9*	117, 92, 108

Key to Positive Controls		Key to Plate Postfix Codes	
SA	Sodium Azide	H	Thinning lawn
DMSO	Dimethyl Sulfoxide	I	Pinpoint colonies

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Amyris (POSF5630)**	0.156 µl	*84.0*	*5.6*	*0.7*	85 H, 78 H, 89 H
		0.313 µl	*60.0*	*26.0*	*0.5*	30 I, 76 I, 74 I
		0.625 µl	*0.0*	*0.0*	*0.0*	0 I, 0 I, 0 I
		1.25 µl	*1.7*	*2.9*	*0.0*	5 I, 0 I, 0 I
		2.5 µl	*0.0*	*0.0*	*0.0*	0 I, 0 I, 0 I
		5 µl	*0.0*	*0.0*	*0.0*	0 I, 0 I, 0 I
	DMSO		*128.0*	*2.6*		126, 127, 131
TA100	**2AN (5% S9)**	2 µg	*4708.3*	*261.8*	*36.8*	5010, 4574, 4541
TA100	**DMSO (+S9)**	–	*144.7*	*2.3*	*1.1*	142, 146, 146

Key to Positive Controls		Key to Plate Postfix Codes	
2AN (5% S9)	2-Aminoanthracene (5% S9)	H	Thinning lawn
DMSO (+S9)	Dimethyl Sulfoxide +S9	I	Pinpoint colonies

Table A-2. R-8 plate counts

Study Name: G343-10 (R-8)
Experiment: Range Finder G343-10 (R-8 POSF5469)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8)
Date Plated: 3/3/2010
Date Counted: 3/5/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**R-8 (POSF5469)**	0.156 µl	*103.7*	*7.1*	*0.9*	105 N, 96 N, 110 N
		0.313 µl	*115.3*	*9.0*	*1.0*	105 N, 121 N, 120 N
		0.625 µl	*104.3*	*7.8*	*0.9*	113 N, 98 N, 102 N
		1.25 µl	*87.7*	*4.6*	*0.8*	85 N, 85 N, 93 N
		2.5 µl	*91.7*	*17.0*	*0.8*	79 N, 111 N, 85 N
		5 µl	*83.0*	*12.1*	*0.7*	81 N, 72 N, 96 N
	DMSO		*114.3*	*4.0*		118, 110, 115
	Untreated Control		*128.2*	*11.4*		134, 109, 130, 129, 139
TA100	**SA**	5 µg	*1510.7*	*33.4*	*13.2*	1495, 1549, 1488
TA100	**DMSO**	–	*105.7*	*12.7*	*0.9*	117, 92, 108

Key to Positive Controls

SA	Sodium Azide
DMSO	Dimethyl Sulfoxide

Key to Plate Postfix Codes

N	Normal background lawn

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**R-8 (POSF5469)**	0.156 µl	*116.7*	*18.6*	*0.9*	119 N, 97 N, 134 N
		0.313 µl	*118.7*	*12.2*	*0.9*	116 N, 108 N, 132 N
		0.625 µl	*119.3*	*7.8*	*0.9*	117 N, 128 N, 113 N
		1.25 µl	*108.7*	*4.5*	*0.8*	113 N, 104 N, 109 N
		2.5 µl	*111.3*	*15.9*	*0.9*	93 N, 120 N, 121 N
		5 µl	*111.3*	*15.3*	*0.9*	108 N, 98 N, 128 N
	DMSO		*128.0*	*2.6*		126, 127, 131
TA100	**2AN (5% S9)**	2 µg	*4708.3*	*261.8*	*36.8*	5010, 4574, 4541
TA100	**DMSO (+S9)**	–	*144.7*	*2.3*	*1.1*	142, 146, 146

Key to Positive Controls

2AN (5% S9)	2-Aminoanthracene (5% S9)
DMSO (+S9)	Dimethyl Sulfoxide +S9

Key to Plate Postfix Codes

N	Normal background lawn

Table A-3. R-8 algae plate counts

Study Name: G343-10 (R-8 algae)
Experiment: Range Finder G343-10 **(R-8 from algae POSF5804)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8 algae)
Date Plated: 3/3/2010
Date Counted: 3/5/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**R-8 from algae (POSF5804)**	0.156 µl	*98.7*	*8.6*	*0.9*	97 N, 91 N, 108 N
		0.313 µl	*101.0*	*3.6*	*0.9*	105 N, 100 N, 98 N
		0.625 µl	*94.0*	*6.1*	*0.8*	101 N, 91 N, 90 N
		1.25 µl	*103.0*	*8.5*	*0.9*	111 N, 94 N, 104 N
		2.5 µl	*89.0*	*5.6*	*0.8*	94 N, 90 N, 83 N
		5 µl	*99.7*	*6.5*	*0.9*	106 P N, 93 P N, 100 P N
	DMSO		*114.3*	*4.0*		118, 110, 115
	Untreated Control		*128.2*	*11.4*		134, 109, 130, 129, 139
TA100	**SA**	5 µg	*1510.7*	*33.4*	*13.2*	1495, 1549, 1488
TA100	**DMSO**	–	*105.7*	*12.7*	*0.9*	117, 92, 108

Key to Positive Controls

SA	Sodium Azide
DMSO	Dimethyl Sulfoxide

Key to Plate Postfix Codes

N	Normal background lawn
P	Precipitate seen as oil like droplets

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**R-8 from algae (POSF5804)**	0.156 µl	*109.3*	*9.8*	*0.9*	98 N, 115 N, 115 N
		0.313 µl	*115.0*	*14.0*	*0.9*	105 N, 131 N, 109 N
		0.625 µl	*119.3*	*7.0*	*0.9*	120 N, 112 N, 126 N
		1.25 µl	*123.0*	*1.7*	*1.0*	124 N, 121 N, 124 N
		2.5 µl	*100.0*	*10.1*	*0.8*	109 N, 102 N, 89 N
		5 µl	*94.3*	*11.2*	*0.7*	104 P N, 97 P N, 82 P N
	DMSO		*128.0*	*2.6*		126, 127, 131
TA100	**2AN (5% S9)**	2 µg	*4708.3*	*261.8*	*36.8*	5010, 4574, 4541
TA100	**DMSO (+S9)**	–	*144.7*	*2.3*	*1.1*	142, 146, 146

Key to Positive Controls

2AN (5% S9)	2-Aminoanthracene (5% S9)
DMSO (+S9)	Dimethyl Sulfoxide +S9

Key to Plate Postfix Codes

N	Normal background lawn
P	Precipitate seen as oil like droplets

Table A-4. S-8 plate counts

Study Name: G343-10 (S-8)
Experiment: Range Finder G343-10 (S-8 POSF4734)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (S-8)
Date Plated: 3/3/2010
Date Counted: 3/5/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**S-8 (POSF4734)**	0.156 µl	*104.3*	*14.6*	*0.9*	94 N, 121 N, 98 N
		0.313 µl	*112.0*	*1.0*	*1.0*	113 N, 111 N, 112 N
		0.625 µl	*108.0*	*3.0*	*0.9*	111 N, 108 N, 105 N
		1.25 µl	*103.3*	*9.0*	*0.9*	112 N, 104 N, 94 N
		2.5 µl	*88.7*	*4.7*	*0.8*	85 N, 87 N, 94 N
		5 µl	*99.0*	*7.5*	*0.9*	91 N, 100 N, 106 N
	DMSO		*114.3*	*4.0*		118, 110, 115
	Untreated Control		*128.2*	*11.4*		134, 109, 130, 129, 139
TA100	**SA**	5 µg	*1510.7*	*33.4*	*13.2*	1495, 1549, 1488
TA100	**DMSO**	–	*105.7*	*12.7*	*0.9*	117, 92, 108

Key to Positive Controls

SA Sodium Azide
DMSO Dimethyl Sulfoxide

Key to Plate Postfix Codes

N Normal background lawn

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**S-8 (POSF4734)**	.156 µl	*126.3*	*11.9*	*1.0*	113 N, 130 N, 136 N
		0.313 µl	*131.0*	*11.5*	*1.0*	132 N, 142 N, 119 N
		0.625 µl	*133.3*	*12.5*	*1.0*	119 N, 139 N, 142 N
		1.25 µl	*132.0*	*10.1*	*1.0*	141 N, 134 N, 121 N
		2.5 µl	*130.3*	*15.0*	*1.0*	131 N, 145 N, 115 N
		5 µl	*124.0*	*5.2*	*1.0*	127 N, 127 N, 118 N
	DMSO		*128.0*	*2.6*		126, 127, 131
TA100	**2AN (5% S9)**	2 µg	*4708.3*	*261.8*	*36.8*	5010, 4574, 4541
TA100	**DMSO (+S9)**	–	*144.7*	*2.3*	*1.1*	142, 146, 146

Key to Positive Controls

2AN (5% S9) 2-Aminoanthracene (5% S9)
DMSO (+S9) Dimethyl Sulfoxide +S9

Key to Plate Postfix Codes

N Normal background lawn

Table A-5. Swedish Biofuel plate counts

Study Name: G343-10 (Swedish)
Experiment: Range Finder G343-10 (**Swedish Biofuel POSF5668**)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Swedish)
Date Plated: 3/3/2010
Date Counted: 3/5/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Swedish Biofuel (POSF5668)**	0.156 µl	*104.7*	*16.1*	*0.9*	123 N, 93 N, 98 N
		0.313 µl	*109.7*	*8.1*	*1.0*	105 N, 105 N, 119 N
		0.625 µl	*94.7*	*4.9*	*0.8*	98 N, 97 N, 89 N
		1.25 µl	*118.7*	*11.6*	*1.0*	132 N, 111 N, 113 N
		2.5 µl	*111.3*	*14.8*	*1.0*	95 N, 124 N, 115 N
		5 µl	*82.3*	*10.8*	*0.7*	70 H, 90 H, 87 H
	DMSO		*114.3*	*4.0*		118, 110, 115
	Untreated Control		*128.2*	*11.4*		134, 109, 130, 129, 139
TA100	**SA**	5 µg	*1510.7*	*33.4*	*13.2*	1495, 1549, 1488
TA100	**DMSO**	–	*105.7*	*12.7*	*0.9*	117, 92, 108

Key to Positive Controls

SA	Sodium Azide
DMSO	Dimethyl Sulfoxide

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	**Swedish Biofuel (POSF5668)**	0.156 µl	*128.0*	*14.7*	*1.0*	112 N, 141 N, 131 N
		0.313 µl	*132.7*	*8.1*	*1.0*	128 N, 128 N, 142 N
		0.625 µl	*133.7*	*12.1*	*1.0*	143 N, 138 N, 120 N
		1.25 µl	*130.7*	*10.0*	*1.0*	142 N, 123 N, 127 N
		2.5 µl	*110.7*	*9.3*	*0.9*	117 N, 115 N, 100 N
		5 µl	*110.0*	*15.6*	*0.9*	102 H, 100 H, 128 H
	DMSO		*128.0*	*2.6*		126, 127, 131
TA100	**2AN (5% S9)**	2 µg	*4708.3*	*261.8*	*36.8*	5010, 4574, 4541
TA100	**DMSO (+S9)**	–	*144.7*	*2.3*	*1.1*	142, 146, 146

Key to Positive Controls

2AN (5% S9)	2-Aminoanthracene (5% S9)
DMSO (+S9)	Dimethyl Sulfoxide +S9

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

APPENDIX E. INDIVIDUAL AND MEAN PLATE COUNTS: FIRST MUTAGENICITY EXPERIMENT WITH AMYRIS, R-8, AND SWEDISH BIOFUEL

Table E-1. Amyris plate counts

Study Name: G343-10 (Amyris)
Experiment: 1st Mutagenicity: G343-10 **Positive Controls**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Amyris)
Date Plated: 3/16/2010
Date Counted: 3/18/2010 to 3/19/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**SA**	5 µg	*2017.7*	*110.8*	*163.6*	2075, 1890, 2088
TA1537	**9AA**	50 µg	*204.0*	*42.7*	*19.1*	175, 253, 184
TA98	**2NF**	5 µg	*1159.0*	*140.8*	*69.5*	1037, 1313, 1127
TA100	**SA**	5 µg	*1781.7*	*89.5*	*16.2*	1885, 1728, 1732
WP2uvrA	**4NQO**	2.5 µg	*2160.7*	*248.0*	*64.2*	2098, 2434, 1950
TA1535	**DMSO**	--	*12.7*	*2.3*	*1.0*	10, 14, 14
TA1537	**DMSO**	--	*7.3*	*2.5*	*0.7*	5, 7, 10
TA98	**DMSO**	--	*23.3*	*7.6*	*1.4*	25, 30, 15
TA100	**DMSO**	--	*123.3*	*7.5*	*1.1*	131, 116, 123
WP2uvrA	**DMSO**	--	*30.0*	*4.0*	*0.9*	34, 30, 26
TA1535*	**SA**	5 µg	*848.0*	*89.4*	*90.9*	783, 811, 950
TA98*	**2NF**	5 µg	*753.0*	*77.9*	*41.8*	720, 697, 842
TA1535*	**DMSO**	--	*8.0*	*2.6*	*0.9*	5, 10, 9
TA98*	**DMSO**	--	*18.7*	*3.1*	*1.0*	22, 16, 18

Key to Positive Controls

SA	Sodium Azide
9AA	9-Aminoacridine hydrochloride
2NF	2-Nitrofluorene
4NQO	4-Nitroquinoline N-oxide
DMSO	Dimethyl Sulfoxide
*	Controls for the re-test on 3/23/10 due to an insufficient number of nontoxic dose levels

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn
I	Pinpoint colonies

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**2AN (5% S9)**	4 µg	*397.0*	*42.0*	*31.3*	415, 427, 349
TA1537	**2AN (5% S9)**	4 µg	*477.3*	*47.5*	*46.2*	515, 493, 424
TA98	**2AN (5% S9)**	2 µg	*2275.3*	*443.7*	*62.6*	2088, 2782, 1956
TA100	**2AN (5% S9)**	2 µg	*3798.0*	*287.2*	*29.7*	4069, 3828, 3497
WP2uvrA	**2AN (5% S9)**	20 µg	*507.7*	*29.4*	*11.9*	528, 521, 474
TA1535	**DMSO (+S9)**	--	*10.0*	*2.0*	*0.8*	8, 12, 10
TA1537	**DMSO (+S9)**	--	*9.7*	*4.5*	*0.9*	14, 5, 10
TA98	**DMSO (+S9)**	--	*35.0*	*2.0*	*1.0*	33, 37, 35
TA100	**DMSO (+S9)**	--	*142.3*	*23.0*	*1.1*	119, 143, 165
WP2uvrA	**DMSO (+S9)**	--	*30.7*	*9.0*	*0.7*	30, 40, 22

Key to Positive Controls

2AN (5% S9)	2-Aminoanthracene (5% S9)
DMSO (+S9)	Dimethyl Sulfoxide +S9

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table E-1 (continued). Amyris plate counts

Study Name: G343-10 (Amyris)
Experiment: 1st Mutagenicity: G343-10 **(Amyris POSF5630)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Amyris)
Date Plated: 3/16/2010
Date Counted: 3/18/2010 to 3/19/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535*	**Amyris (POSF5630)**	0.0013 µl	*11.3*	*4.5*	*1.2*	11, 7, 16
		0.0025 µl	*11.7*	*5.5*	*1.2*	8, 9, 18
		0.005 µl	*11.7*	*2.1*	*1.2*	10, 11, 14
		0.01 µl	*12.0*	*3.0*	*1.3*	12 N, 15 N, 9 N
		0.02 µl	*9.0*	*3.0*	*1.0*	12 H, 6 H, 9 H
		0.039 µl	*7.7*	*2.9*	*0.8*	6 H, 6 H, 11 H
	DMSO		*9.3*	*2.5*		7, 12, 9
	Untreated Control		*8.6*	*2.7*		7, 12, 9, 10, 5
TA1537	**Amyris (POSF5630)**	0.005 µl	*8.7*	*3.2*	*0.8*	10, 5, 11
		0.01 µl	*7.7*	*2.5*	*0.7*	8, 10, 5
		0.02 µl	*6.3*	*3.2*	*0.6*	4 N, 5 N, 10 N
		0.039 µl	*7.7*	*2.5*	*0.7*	5 H, 8 H, 10 H
		0.078 µl	*3.0*	*2.0*	*0.3*	1 H, 3 H, 5 H
		0.156 µl	*1.3*	*1.5*	*0.1*	1 H, 0 H, 3 H
	DMSO		*10.7*	*3.1*		8, 14, 10
	Untreated Control		*10.2*	*2.9*		10, 8, 7, 12, 14
TA98*	**Amyris (POSF5630)**	0.0013 µl	*21.3*	*10.7*	*1.2*	19, 12, 33
		0.0025 µl	*20.7*	*4.6*	*1.1*	18, 18, 26
		0.005 µl	*18.3*	*6.7*	*1.0*	26, 15, 14
		0.01 µl	*13.0*	*1.7*	*0.7*	12 N, 12 N, 15 N
		0.02 µl	*15.3*	*4.0*	*0.9*	19 H, 16 H, 11 H
		0.039 µl	*13.0*	*1.7*	*0.7*	12 H, 15 H, 12 H
	DMSO		*18.0*	*4.0*		18, 14, 22
	Untreated Control		*18.0*	*4.1*		12, 23, 20, 18, 17
TA100	**Amyris (POSF5630)**	0.005 µl	*106.0*	*14.1*	*1.0*	104, 121, 93
		0.01 µl	*112.3*	*12.7*	*1.0*	127, 104, 106
		0.02 µl	*97.3*	*2.5*	*0.9*	95 N, 97 N, 100 N
		0.039 µl	*89.0*	*14.1*	*0.8*	74 H, 91 H, 102 H
		0.078 µl	*74.3*	*9.7*	*0.7*	85 H, 66 H, 72 H
		0.156 µl	*85.3*	*6.4*	*0.8*	78 H, 89 H, 89 H
	DMSO		*109.7*	*8.1*		106, 119, 104
	Untreated Control		*137.4*	*17.5*		143, 124, 164, 120, 136
WP2uvrA	**Amyris (POSF5630)**	0.005 µl	*26.3*	*4.0*	*0.8*	30, 22, 27
		0.01 µl	*29.3*	*3.5*	*0.9*	26, 29, 33
		0.02 µl	*26.3*	*3.5*	*0.8*	26, 23, 30
		0.039 µl	*31.0*	*8.7*	*0.9*	25, 41, 27
		0.078 µl	*24.0*	*2.6*	*0.7*	22, 23, 27
		0.156 µl	*27.0*	*5.6*	*0.8*	22 N, 26 N, 33 N
	DMSO		*33.7*	*5.7*		29, 40, 32
	Untreated Control		*28.2*	*7.4*		23, 30, 38, 31, 19

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn
I	Pinpoint colonies

* Strains re-tested with different dose levels on 3/23/10 due to an insufficient number of nontoxic dose levels

Table E-1 (continued). Amyris plate counts

Study Name: G343-10 (Amyris)
Experiment: 1st Mutagenicity: G343-10 **(Amyris POSF5630)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Amyris)
Date Plated: 3/16/2010
Date Counted: 3/18/2010 to 3/19/2010

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**Amyris (POSF5630)**	0.005 µl	*10.3*	*4.7*	*0.8*	5, 14, 12
		0.01 µl	*9.7*	*5.7*	*0.8*	5, 16, 8
		0.02 µl	*6.3*	*3.2*	*0.5*	10 N, 4 N, 5 N
		0.039 µl	*7.0*	*1.7*	*0.6*	8 H, 8 H, 5 H
		0.078 µl	*7.3*	*3.1*	*0.6*	10 H, 8 H, 4 H
		0.156 µl	*6.7*	*2.1*	*0.5*	6 H, 5 H, 9 H
	DMSO		*12.7*	*5.7*		11, 8, 19
TA1537	**Amyris (POSF5630)**	0.005 µl	*8.3*	*3.5*	*0.8*	5, 8, 12
		0.01 µl	*6.0*	*1.7*	*0.6*	5, 8, 5
		0.02 µl	*7.7*	*0.6*	*0.7*	8 N, 7 N, 8 N
		0.039 µl	*8.0*	*3.6*	*0.8*	12 H, 5 H, 7 H
		0.078 µl	*6.0*	*1.7*	*0.6*	5 H, 8 H, 5 H
		0.156 µl	*6.3*	*4.2*	*0.6*	11 H, 3 H, 5 H
	DMSO		*10.3*	*2.1*		8, 12, 11
TA98	**Amyris (POSF5630)**	0.005 µl	*31.0*	*5.2*	*0.9*	34, 34, 25
		0.01 µl	*32.0*	*5.2*	*0.9*	29, 29, 38
		0.02 µl	*28.7*	*3.8*	*0.8*	33, 26, 27
		0.039 µl	*22.3*	*5.8*	*0.6*	29 N, 19 N, 19 N
		0.078 µl	*30.7*	*2.1*	*0.8*	30 H, 29 H, 33 H
		0.156 µl	*21.7*	*3.5*	*0.6*	25 H, 22 H, 18 H
	DMSO		*36.3*	*8.5*		33, 30, 46
TA100	**Amyris (POSF5630)**	0.005 µl	*132.0*	*26.1*	*1.0*	157, 105, 134
		0.01 µl	*131.7*	*17.0*	*1.0*	131, 115, 149
		0.02 µl	*117.7*	*7.4*	*0.9*	115 N, 126 N, 112 N
		0.039 µl	*106.7*	*7.8*	*0.8*	113 H, 98 H, 109 H
		0.078 µl	*103.7*	*8.1*	*0.8*	100 H, 113 H, 98 H
		0.156 µl	*101.3*	*4.2*	*0.8*	106 H, 98 H, 100 H
	DMSO		*128.0*	*9.8*		117, 131, 136
WP2uvrA	**Amyris (POSF5630)**	0.005 µl	*28.7*	*9.3*	*0.7*	18, 35, 33
		0.01 µl	*30.3*	*11.9*	*0.7*	44, 25, 22
		0.02 µl	*32.7*	*2.3*	*0.8*	30, 34, 34
		0.039 µl	*32.0*	*6.6*	*0.8*	38, 33, 25
		0.078 µl	*33.3*	*3.5*	*0.8*	33, 37, 30
		0.156 µl	*36.0*	*2.6*	*0.8*	37 N, 38 N, 33 N
	DMSO		*42.7*	*2.5*		43, 45, 40

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table E-2. R-8 plate counts

Study Name: G343-10 (R-8)
Experiment: 1st Mutagenicity: G343-10 **(R-8 POSF5469)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8)
Date Plated: 3/16/2010
Date Counted: 3/18/2010 to 3/19/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**R-8 (POSF5469)**	0.156 µl	*8.7*	*5.0*	*0.7*	8, 4, 14
		0.313 µl	*14.0*	*1.7*	*1.1*	12, 15, 15
		0.625 µl	*16.3*	*11.2*	*1.3*	12, 8, 29
		1.25 µl	*14.7*	*5.0*	*1.2*	10, 14, 20
		2.5 µl	*14.7*	*5.8*	*1.2*	18, 8, 18
		5 µl	*15.0*	*1.0*	*1.2*	16 N, 15 N, 14 N
	DMSO		*12.3*	*4.7*		7, 16, 14
	Untreated Control		*14.2*	*3.1*		11, 19, 14, 15, 12
TA1537	**R-8 (POSF5469)**	0.156 µl	*9.0*	*3.6*	*0.8*	12, 5, 10
		0.313 µl	*7.3*	*4.0*	*0.7*	5, 5, 12
		0.625 µl	*9.0*	*4.4*	*0.8*	11, 4, 12
		1.25 µl	*7.0*	*0.0*	*0.7*	7, 7, 7
		2.5 µl	*5.3*	*1.5*	*0.5*	7, 5, 4
		5 µl	*6.3*	*2.1*	*0.6*	4 N, 8 N, 7 N
	DMSO		*10.7*	*3.1*		8, 14, 10
	Untreated Control		*10.2*	*2.9*		10, 8, 7, 12, 14
TA98	**R-8 (POSF5469)**	0.156 µl	*24.0*	*4.6*	*1.4*	20, 23, 29
		0.313 µl	*21.0*	*7.2*	*1.3*	15, 29, 19
		0.625 µl	*20.7*	*3.8*	*1.2*	25, 18, 19
		1.25 µl	*18.7*	*3.1*	*1.1*	18, 22, 16
		2.5 µl	*18.0*	*1.7*	*1.1*	16 N, 19 N, 19 N
		5 µl	*16.3*	*5.1*	*1.0*	22 N, 15 H, 12 H
	DMSO		*16.7*	*3.1*		16, 14, 20
	Untreated Control		*20.6*	*7.1*		18, 15, 18, 19, 33
TA100	**R-8 (POSF5469)**	0.156 µl	*112.0*	*9.6*	*1.0*	123, 108, 105
		0.313 µl	*108.7*	*3.5*	*1.0*	112, 105, 109
		0.625 µl	*101.0*	*12.8*	*0.9*	115, 90, 98
		1.25 µl	*108.0*	*3.0*	*1.0*	108, 111, 105
		2.5 µl	*110.0*	*14.0*	*1.0*	100, 104, 126
		5 µl	*109.3*	*15.3*	*1.0*	96 N, 106 N, 126 N
	DMSO		*109.7*	*8.1*		106, 119, 104
	Untreated Control		*137.4*	*17.5*		143, 124, 164, 120, 136
WP2uvrA	**R-8 (POSF5469)**	0.156 µl	*32.7*	*11.9*	*1.0*	41, 19, 38
		0.313 µl	*29.3*	*3.5*	*0.9*	29, 33, 26
		0.625 µl	*30.7*	*10.7*	*0.9*	40, 19, 33
		1.25 µl	*26.3*	*6.4*	*0.8*	31, 29, 19
		2.5 µl	*35.0*	*6.2*	*1.0*	33, 30, 42
		5 µl	*27.7*	*4.9*	*0.8*	22 N, 31 N, 30 N
	DMSO		*33.7*	*5.7*		29, 40, 32
	Untreated Control		*28.2*	*7.4*		23, 30, 38, 31, 19

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table E-2 (continued). R-8 plate counts

Study Name: G343-10 (R-8)
Experiment: 1st Mutagenicity: G343-10 **(R-8 POSF5469)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8)
Date Plated: 3/16/2010
Date Counted: 3/18/2010 to 3/19/2010

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**R-8 (POSF5469)**	0.156 µl	*9.0*	*1.7*	*0.7*	8, 11, 8
		0.313 µl	*6.7*	*1.5*	*0.5*	8, 7, 5
		0.625 µl	*12.3*	*3.8*	*1.0*	14, 8, 15
		1.25 µl	*9.7*	*3.8*	*0.8*	8, 14, 7
		2.5 µl	*11.0*	*3.0*	*0.9*	8, 11, 14
		5 µl	*17.3*	*4.2*	*1.4*	16 N, 14 N, 22 N
	DMSO		*12.7*	*5.7*		11, 8, 19
TA1537	**R-8 (POSF5469)**	0.156 µl	*13.3*	*4.9*	*1.3*	10, 19, 11
		0.313 µl	*8.7*	*2.1*	*0.8*	8, 11, 7
		0.625 µl	*12.0*	*4.4*	*1.2*	14, 15, 7
		1.25 µl	*11.0*	*3.0*	*1.1*	11, 8, 14
		2.5 µl	*9.0*	*2.6*	*0.9*	8, 7, 12
		5 µl	*9.7*	*5.1*	*0.9*	4 N, 11 N, 14 N
	DMSO		*10.3*	*2.1*		8, 12, 11
TA98	**R-8 (POSF5469)**	0.156 µl	*26.7*	*4.0*	*0.7*	29, 22, 29
		0.313 µl	*31.7*	*4.6*	*0.9*	29, 29, 37
		0.625 µl	*32.3*	*7.8*	*0.9*	26, 30, 41
		1.25 µl	*32.7*	*2.5*	*0.9*	30, 35, 33
		2.5 µl	*30.3*	*0.6*	*0.8*	30, 30, 31
		5 µl	*29.3*	*4.0*	*0.8*	27 N, 27 N, 34 N
	DMSO		*36.3*	*8.5*		33, 30, 46
TA100	**R-8 (POSF5469)**	0.156 µl	*99.3*	*3.1*	*0.8*	102, 96, 100
		0.313 µl	*111.3*	*11.7*	*0.9*	124, 101, 109
		0.625 µl	*114.3*	*6.1*	*0.9*	121, 113, 109
		1.25 µl	*100.0*	*12.1*	*0.8*	102, 111, 87
		2.5 µl	*107.0*	*12.8*	*0.8*	104, 96, 121
		5 µl	*101.3*	*10.4*	*0.8*	93 N, 113 N, 98 N
	DMSO		*128.0*	*9.8*		117, 131, 136
WP2uvrA	**R-8 (POSF5469)**	0.156 µl	*30.7*	*6.4*	*0.7*	38, 27, 27
		0.313 µl	*27.3*	*2.5*	*0.6*	30, 27, 25
		0.625 µl	*29.0*	*1.7*	*0.7*	30, 27, 30
		1.25 µl	*33.7*	*7.1*	*0.8*	35, 26, 40
		2.5 µl	*37.3*	*5.9*	*0.9*	35, 33, 44
		5 µl	*31.3*	*5.1*	*0.7*	27 N, 30 N, 37 N
	DMSO		*42.7*	*2.5*		43, 45, 40

Key to Plate Postfix Codes

N Normal background lawn

Table E-3. Swedish Biofuel plate counts

Study Name: G343-10 (Swedish)
Experiment: 1st Mutagenicity: G343-10 **(Swedish Biofuel POSF5668)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Swedish)
Date Plated: 3/16/2010
Date Counted: 3/18/2010 to 3/19/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	Swedish Biofuel (POSF5668)	0.156 µl	14.3	4.0	1.2	12, 19, 12
		0.313 µl	9.3	5.9	0.8	7, 5, 16
		0.625 µl	11.0	4.0	0.9	15, 11, 7
		1.25 µl	9.3	3.8	0.8	11, 5, 12
		2.5 µl	8.7	2.1	0.7	7 N, 11 N, 8 N
		5 µl	7.3	3.1	0.6	4 H, 8 H, 10 H
	DMSO		12.3	4.7		7, 16, 14
	Untreated Control		14.2	3.1		11, 19, 14, 15, 12
TA1537	Swedish Biofuel (POSF5668)	0.156 µl	10.7	1.2	1.0	10, 12, 10
		0.313 µl	8.3	1.5	0.8	10, 7, 8
		0.625 µl	8.3	3.5	0.8	12, 5, 8
		1.25 µl	5.0	1.0	0.5	5, 4, 6
		2.5 µl	6.7	3.5	0.6	3 N, 10 N, 7 N
		5 µl	2.3	1.5	0.2	1 H, 2 H, 4 H
	DMSO		10.7	3.1		8, 14, 10
	Untreated Control		10.2	2.9		10, 8, 7, 12, 14
TA98	Swedish Biofuel (POSF5668)	0.156 µl	25.0	7.9	1.5	34, 19, 22
		0.313 µl	20.7	4.6	1.2	26, 18, 18
		0.625 µl	19.3	1.2	1.2	18, 20, 20
		1.25 µl	21.7	5.8	1.3	15, 25, 25
		2.5 µl	17.0	8.9	1.0	27 N, 10 N, 14 N
		5 µl	14.7	3.5	0.9	11 H, 18 H, 15 H
	DMSO		16.7	3.1		16, 14, 20
	Untreated Control		20.6	7.1		18, 15, 18, 19, 33
TA100	Swedish Biofuel (POSF5668)	0.156 µl	119.7	15.4	1.1	102, 130, 127
		0.313 µl	117.3	4.6	1.1	112, 120, 120
		0.625 µl	107.3	4.7	1.0	111, 102, 109
		1.25 µl	105.7	13.7	1.0	90, 115, 112
		2.5 µl	82.3	7.2	0.8	74 N, 86 N, 87 N
		5 µl	90.0	13.0	0.8	97 H, 75 H, 98 H
	DMSO		109.7	8.1		106, 119, 104
	Untreated Control		137.4	17.5		143, 124, 164, 120, 136
WP2uvrA	Swedish Biofuel (POSF5668)	0.156 µl	26.7	0.6	0.8	27, 27, 26
		0.313 µl	23.7	4.2	0.7	19, 27, 25
		0.625 µl	27.0	6.2	0.8	25, 22, 34
		1.25 µl	31.7	5.0	0.9	37, 27, 31
		2.5 µl	27.7	7.8	0.8	30 N, 34 N, 19 N
		5 µl	12.3	3.5	0.4	16 H, 12 H, 9 H
	DMSO		33.7	5.7		29, 40, 32
	Untreated Control		28.2	7.4		23, 30, 38, 31, 19

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table E-3 (continued). Swedish Biofuel plate counts

Study Name: G343-10 (Swedish)
Experiment: 1st Mutagenicity: G343-10 **(Swedish Biofuel POSF5668)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Swedish)
Date Plated: 3/16/2010
Date Counted: 3/18/2010 to 3/19/2010

With metabolic activation (5%S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**Swedish Biofuel (POSF5668)**	0.156 µl	*12.7*	*4.2*	*1.0*	8, 14, 16
		0.313 µl	*12.0*	*2.6*	*0.9*	11, 10, 15
		0.625 µl	*9.7*	*3.8*	*0.8*	14, 8, 7
		1.25 µl	*11.3*	*9.5*	*0.9*	22, 8, 4
		2.5 µl	*3.7*	*1.2*	*0.3*	5 N, 3 N, 3 N
		5 µl	*4.3*	*2.1*	*0.3*	6 H, 2 H, 5 H
	DMSO		*12.7*	*5.7*		11, 8, 19
TA1537	**Swedish Biofuel (POSF5668)**	0.156 µl	*7.7*	*3.1*	*0.7*	5, 7, 11
		0.313 µl	*6.0*	*3.6*	*0.6*	3, 5, 10
		0.625 µl	*5.3*	*2.5*	*0.5*	8, 3, 5
		1.25 µl	*8.0*	*3.6*	*0.8*	5 N, 12 N, 7 N
		2.5 µl	*8.3*	*2.9*	*0.8*	10 H, 10 H, 5 H
		5 µl	*0.0*	*0.0*	*0.0*	0 I, 0 I, 0 I
	DMSO		*10.3*	*2.1*		8, 12, 11
TA98	**Swedish Biofuel (POSF5668)**	0.156 µl	*28.7*	*6.5*	*0.8*	22, 29, 35
		0.313 µl	*33.3*	*0.6*	*0.9*	33, 34, 33
		0.625 µl	*32.3*	*8.6*	*0.9*	23, 34, 40
		1.25 µl	*30.7*	*2.9*	*0.8*	34, 29, 29
		2.5 µl	*26.0*	*9.8*	*0.7*	29 N, 34 N, 15 N
		5 µl	*29.0*	*6.9*	*0.8*	25 H, 25 H, 37 H
	DMSO		*36.3*	*8.5*		33, 30, 46
TA100	**Swedish Biofuel (POSF5668)**	0.156 µl	*142.7*	*10.2*	*1.1*	147, 131, 150
		0.313 µl	*132.0*	*19.7*	*1.0*	111, 135, 150
		0.625 µl	*135.0*	*9.6*	*1.1*	128, 146, 131
		1.25 µl	*120.3*	*26.1*	*0.9*	123, 145, 93
		2.5 µl	*119.3*	*13.3*	*0.9*	126 N, 104 N, 128 N
		5 µl	*116.3*	*18.0*	*0.9*	130 H, 123 H, 96 H
	DMSO		*128.0*	*9.8*		117, 131, 136
WP2uvrA	**Swedish Biofuel (POSF5668)**	0.156 µl	*38.0*	*7.9*	*0.9*	44, 29, 41
		0.313 µl	*38.7*	*5.0*	*0.9*	44, 38, 34
		0.625 µl	*36.7*	*3.5*	*0.9*	33, 37, 40
		1.25 µl	*34.7*	*9.0*	*0.8*	26, 34, 44
		2.5 µl	*30.3*	*4.2*	*0.7*	29 N, 35 N, 27 N
		5 µl	*35.7*	*8.1*	*0.8*	45 H, 31 H, 31 H
	DMSO		*42.7*	*2.5*		43, 45, 40

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn
I	Pinpoint colonies

APPENDIX F. INDIVIDUAL AND MEAN PLATE COUNTS: FIRST MUTAGENICITY EXPERIMENT WITH R-8 FROM ALGAE AND S-8

Table F-1. R-8 from algae plate counts

Study Name: G343-10 (R-8 algae)
Experiment: 1st Mutagenicity: G343-10 **Positive Controls**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8 algae)
Date Plated: 3/17/2010
Date Counted: 3/19/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**SA**	5 µg	*2189.7*	*41.1*	*142.8*	2150, 2232, 2187
TA1537	**9AA**	50 µg	*205.3*	*32.8*	*30.8*	243, 190, 183
TA98	**2NF**	5 µg	*1324.7*	*129.1*	*41.0*	1295, 1466, 1213
TA100	**SA**	5 µg	*1960.3*	*60.5*	*15.8*	1978, 1893, 2010
WP2uvrA	**4NQO**	2.5 µg	*3400.0*	*230.8*	*99.0*	3329, 3213, 3658
TA1535	**DMSO**	–	*25.7*	*1.5*	*1.5*	25, 24, 22
TA1537	**DMSO**	–	*10.0*	*4.6*	*1.5*	5, 14, 11
TA98	**DMSO**	–	*26.7*	*7.2*	*0.8*	22, 35, 23
TA100	**DMSO**	–	*125.7*	*14.6*	*1.0*	141, 112, 124
WP2uvrA	**DMSO**	–	*31.0*	*9.5*	*0.9*	22, 41, 30

Key to Positive Controls

SA	Sodium Azide
9AA	9-Aminoacridine hydrochloride
2NF	2-Nitrofluorene
4NQO	4-Nitroquinoline N-oxide
DMSO	Dimethyl Sulfoxide

Key to Plate Postfix Codes

P	Precipitate
N	Normal background lawn

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**2AN (5% S9)**	4 µg	*382.3*	*34.2*	*32.8*	346, 414, 387
TA1537	**2AN (5% S9)**	4 µg	*419.0*	*40.0*	*43.3*	383, 462, 412
TA98	**2AN (5% S9)**	2 µg	*1821.3*	*733.1*	*49.2*	2120, 986, 2358
TA100	**2AN (5% S9)**	2 µg	*3344.7*	*296.6*	*27.1*	3215, 3684, 3135
WP2uvrA	**2AN (5% S9)**	20 µg	*685.7*	*93.0*	*20.6*	793, 633, 631
TA1535	**DMSO (+S9)**	–	*13.3*	*2.9*	*1.1*	15, 10, 15
TA1537	**DMSO (+S9)**	–	*8.7*	*2.1*	*0.9*	8, 7, 11
TA98	**DMSO (+S9)**	–	*37.7*	*5.5*	*1.0*	35, 44, 34
TA100	**DMSO (+S9)**	–	*122.3*	*21.6*	*1.0*	120, 102, 145
WP2uvrA	**DMSO (+S9)**	–	*35.0*	*4.6*	*1.0*	34, 40, 31

Key to Positive Controls

2AN (5% S9)	2-Aminoanthracene (5% S9)
DMSO (+S9)	Dimethyl Sulfoxide +S9

Key to Plate Postfix Codes

P	Precipitate
N	Normal background lawn

Table F-1 (continued). R-8 from algae plate counts

Study Name: G343-10 (R-8 algae)
Experiment: 1st Mutagenicity: G343-10 (R-8 from algae POSF5804)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8 algae)
Date Plated: 3/17/2010
Date Counted: 3/19/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**R-8 from algae (POSF5804)**	0.156 µl	*12.0*	*5.6*	*0.8*	7, 11, 18
		0.313 µl	*15.3*	*3.1*	*1.0*	12, 18, 16
		0.625 µl	*10.3*	*2.1*	*0.7*	8, 12, 11
		1.25 µl	*16.7*	*4.2*	*1.1*	20, 18, 12
		2.5 µl	*11.3*	*2.3*	*0.7*	10, 10, 14
		5 µl	*10.7*	*4.6*	*0.7*	16 PN, 8 PN, 8 PN
	DMSO		*15.3*	*4.5*		20, 15, 11
	Untreated Control		*17.6*	*5.6*		19, 12, 25, 12, 20
TA1537	**R-8 from algae (POSF5804)**	0.156 µl	*6.3*	*3.2*	*1.0*	4, 5, 10
		0.313 µl	*7.0*	*1.7*	*1.1*	8, 5, 8
		0.625 µl	*6.7*	*2.9*	*1.0*	5, 5, 10
		1.25 µl	*4.7*	*0.6*	*0.7*	5, 4, 5
		2.5 µl	*11.0*	*3.0*	*1.7*	8, 14, 11
		5 µl	*7.0*	*1.7*	*1.1*	8 PN, 5 PN, 8 PN
	DMSO		*6.7*	*3.8*		4, 11, 5
	Untreated Control		*11.2*	*4.4*		16, 8, 8, 8, 16
TA98	**R-8 from algae (POSF5804)**	0.156 µl	*21.7*	*3.5*	*0.7*	22, 18, 25
		0.313 µl	*24.7*	*2.3*	*0.8*	26, 22, 26
		0.625 µl	*24.0*	*6.6*	*0.7*	18, 23, 31
		1.25 µl	*23.7*	*1.2*	*0.7*	23, 25, 23
		2.5 µl	*24.7*	*9.1*	*0.8*	15, 33, 26
		5 µl	*18.0*	*4.4*	*0.6*	15 PN, 23 PN, 16 PN
	DMSO		*32.3*	*2.1*		30, 33, 34
	Untreated Control		*24.4*	*6.6*		20, 33, 30, 19, 20
TA100	**R-8 from algae (POSF5804)**	0.156 µl	*112.7*	*5.7*	*0.9*	108, 111, 119
		0.313 µl	*106.0*	*14.0*	*0.9*	120, 92, 106
		0.625 µl	*111.7*	*8.1*	*0.9*	121, 108, 106
		1.25 µl	*97.0*	*4.0*	*0.8*	97, 101, 93
		2.5 µl	*98.3*	*22.1*	*0.8*	74, 117, 104
		5 µl	*91.3*	*9.1*	*0.7*	90 PN, 83 PN, 101 PN
	DMSO		*124.0*	*9.8*		113, 127, 132
	Untreated Control		*116.4*	*10.8*		130, 106, 126, 109, 111

Table F-1 (continued). R-8 from algae plate counts

Study Name: G343-10 (R-8 algae)
Experiment: 1st Mutagenicity: G343-10 **(R-8 from algae POSF5804)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8 algae)
Date Plated: 3/17/2010
Date Counted: 3/19/2010

Without metabolic activation

WP2uvrA	**R-8 from algae (POSF5804)**	0.156 µl	*20.3*	*3.8*	*0.6*	23, 16, 22
		0.313 µl	*25.7*	*12.9*	*0.7*	22, 15, 40
		0.625 µl	*26.3*	*6.4*	*0.8*	29, 31, 19
		1.25 µl	*27.3*	*3.8*	*0.8*	30, 23, 29
		2.5 µl	*33.3*	*6.4*	*1.0*	37, 37, 26
		5 µl	*32.3*	*5.0*	*0.9*	37 P N, 33 P N, 27 P N
	DMSO		*34.3*	*8.0*		35, 26, 42
	Untreated Control		*34.2*	*5.8*		31, 31, 30, 44, 35

Key to Plate Postfix Codes

P	Precipitate seen as oil like droplets
N	Normal background lawn

Table F-1 (continued). R-8 from algae plate counts

Study Name: G343-10 (R-8 algae)
Experiment: 1st Mutagenicity: G343-10 **(R-8 from algae POSF5804)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8 algae)
Date Plated: 3/17/2010
Date Counted: 3/19/2010

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**R-8 from algae (POSF5804)**	0.156 µl	*9.7*	*5.7*	*0.8*	8, 5, 16
		0.313 µl	*11.3*	*4.5*	*1.0*	7, 11, 16
		0.625 µl	*12.3*	*5.1*	*1.1*	8, 18, 11
		1.25 µl	*10.0*	*4.0*	*0.9*	6, 10, 14
		2.5 µl	*10.0*	*2.0*	*0.9*	10, 8, 12
		5 µl	*16.0*	*2.6*	*1.4*	14 P N, 15 P N, 19 P N
	DMSO		*11.7*	*6.5*		18, 12, 5
TA1537	**R-8 from algae (POSF5804)**	0.156 µl	*12.0*	*1.7*	*1.2*	14, 11, 11
		0.313 µl	*8.0*	*1.7*	*0.8*	7, 10, 7
		0.625 µl	*12.3*	*2.5*	*1.3*	15, 10, 12
		1.25 µl	*7.7*	*5.5*	*0.8*	4, 14, 5
		2.5 µl	*9.7*	*4.5*	*1.0*	14, 10, 5
		5 µl	*8.7*	*1.5*	*0.9*	10 P N, 9 P N, 7 P N
	DMSO		*9.7*	*3.8*		14, 7, 8
TA98	**R-8 from algae (POSF5804)**	0.156 µl	*32.7*	*2.3*	*0.9*	30, 34, 34
		0.313 µl	*35.0*	*6.6*	*0.9*	29, 34, 42
		0.625 µl	*29.7*	*2.5*	*0.8*	32, 27, 30
		1.25 µl	*33.7*	*4.0*	*0.9*	33, 30, 38
		2.5 µl	*28.7*	*3.2*	*0.8*	31, 25, 30
		5 µl	*36.3*	*5.1*	*1.0*	35 P N, 42 P N, 32 P N
	DMSO		*37.0*	*2.6*		38, 34, 39
TA100	**R-8 from algae (POSF5804)**	0.156 µl	*115.7*	*9.3*	*0.9*	108, 113, 126
		0.313 µl	*116.7*	*10.0*	*0.9*	109, 128, 113
		0.625 µl	*113.7*	*15.6*	*0.9*	128, 116, 97
		1.25 µl	*105.7*	*15.0*	*0.9*	98, 96, 123
		2.5 µl	*115.3*	*10.5*	*0.9*	105, 126, 115
		5 µl	*100.3*	*7.5*	*0.8*	93 P N, 108 P N, 100 P N
	DMSO		*123.3*	*21.9*		136, 98, 136
WP2uvrA	**R-8 from algae (POSF5804)**	0.156 µl	*35.3*	*6.1*	*1.1*	34, 30, 42
		0.313 µl	*33.0*	*6.1*	*1.0*	40, 30, 29
		0.625 µl	*35.0*	*4.6*	*1.0*	30, 39, 36
		1.25 µl	*31.3*	*5.5*	*0.9*	26, 31, 37
		2.5 µl	*30.3*	*6.4*	*0.9*	33, 23, 35
		5 µl	*36.0*	*8.5*	*1.1*	44 P N, 37 P N, 27 P N
	DMSO		*33.3*	*5.9*		29, 31, 40

Key to Plate Postfix Codes

P	Precipitate seen as oil like droplets
N	Normal background lawn

Table F-2. S-8 plate counts

Study Name: G343-10 (S-8)
Experiment: 1st Mutagenicity: G343-10 (S-8 POSF4734)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (S-8)
Date Plated: 3/17/2010
Date Counted: 3/19/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**S-8 (POSF4734)**	0.156 µl	*24.7*	*5.7*	*1.6*	23, 31, 20
		0.313 µl	*16.7*	*5.7*	*1.1*	23, 15, 12
		0.625 µl	*17.0*	*7.2*	*1.1*	25, 15, 11
		1.25 µl	*23.7*	*8.6*	*1.5*	22, 33, 16
		2.5 µl	*13.7*	*5.5*	*0.9*	14, 8, 19
		5 µl	*14.0*	*6.0*	*0.9*	8 N, 14 N, 20 N
	DMSO		*15.3*	*4.5*		20, 15, 11
	Untreated Control		*17.6*	*5.6*		19, 12, 25, 12, 20
TA1537	**S-8 (POSF4734)**	0.156 µl	*7.7*	*2.5*	*1.1*	5, 8, 10
		0.313 µl	*5.3*	*2.5*	*0.8*	5, 3, 8
		0.625 µl	*7.0*	*3.6*	*1.1*	11, 6, 4
		1.25 µl	*6.7*	*2.5*	*1.0*	7, 4, 9
		2.5 µl	*7.7*	*0.6*	*1.1*	8, 7, 8
		5 µl	*6.7*	*3.5*	*1.0*	10 N, 3 N, 7 N
	DMSO		*6.7*	*3.8*		4, 11, 5
	Untreated Control		*11.2*	*4.4*		16, 8, 8, 8, 16
TA98	**S-8 (POSF4734)**	0.156 µl	*26.0*	*4.6*	*0.8*	22, 25, 31
		0.313 µl	*23.7*	*6.8*	*0.7*	16, 26, 29
		0.625 µl	*27.7*	*5.0*	*0.9*	23, 33, 27
		1.25 µl	*34.0*	*4.0*	*1.1*	38, 34, 30
		2.5 µl	*21.7*	*7.4*	*0.7*	16, 19, 30
		5 µl	*30.3*	*2.3*	*0.9*	29 N, 29 N, 33 N
	DMSO		*32.3*	*2.1*		30, 33, 34
	Untreated Control		*24.4*	*6.6*		20, 33, 30, 19, 20
TA100	**S-8 (POSF4734)**	0.156 µl	*120.3*	*13.1*	*1.0*	119, 108, 134
		0.313 µl	*109.0*	*13.0*	*0.9*	101, 102, 124
		0.625 µl	*114.0*	*21.7*	*0.9*	101, 102, 139
		1.25 µl	*117.7*	*15.9*	*0.9*	109, 136, 108
		2.5 µl	*120.0*	*21.6*	*1.0*	96, 126, 138
		5 µl	*116.0*	*14.5*	*0.9*	115 N, 102 N, 131 N
	DMSO		*124.0*	*9.8*		113, 127, 132
	Untreated Control		*116.4*	*10.8*		130, 106, 126, 109, 111
WP2uvrA	**S-8 (POSF4734)**	0.156 µl	*33.3*	*5.8*	*1.0*	30, 40, 30
		0.313 µl	*32.3*	*6.7*	*0.9*	25, 38, 34
		0.625 µl	*32.7*	*12.1*	*1.0*	34, 44, 20
		1.25 µl	*39.0*	*2.6*	*1.1*	38, 37, 42
		2.5 µl	*37.0*	*7.9*	*1.1*	28, 43, 40
		5 µl	*38.7*	*5.0*	*1.1*	34 N, 44 N, 38 N
	DMSO		*34.3*	*8.0*		35, 26, 42
	Untreated Control		*34.2*	*5.8*		31, 31, 30, 44, 35

Key to Plate Postfix Codes

N Normal background lawn

Table F-2 (continued). S-8 plate counts

Study Name: G343-10 (S-8)
Experiment: 1st Mutagenicity: G343-10 (S-8 POSF4734)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (S-8)
Date Plated: 3/17/2010
Date Counted: 3/19/2010

With metabolic activation (5% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**S-8 (POSF4734)**	0.156 µl	*11.0*	*3.6*	*0.9*	14, 7, 12
		0.313 µl	*9.0*	*2.6*	*0.8*	12, 8, 7
		0.625 µl	*11.7*	*3.1*	*1.0*	9, 11, 15
		1.25 µl	*11.3*	*4.5*	*1.0*	11, 7, 16
		2.5 µl	*13.7*	*3.8*	*1.2*	12, 11, 18
		5 µl	*23.0*	*12.3*	*2.0*	18 N, 14 N, 37 N
	DMSO		*11.7*	*6.5*		18, 12, 5
TA1537	**S-8 (POSF4734)**	0.156 µl	*11.7*	*3.5*	*1.2*	15, 8, 12
		0.313 µl	*14.3*	*4.0*	*1.5*	12, 12, 19
		0.625 µl	*12.3*	*2.9*	*1.3*	9, 14, 14
		1.25 µl	*9.7*	*2.9*	*1.0*	8, 13, 8
		2.5 µl	*12.7*	*2.1*	*1.3*	12, 15, 11
		5 µl	*9.7*	*1.5*	*1.0*	11 N, 10 N, 8 N
	DMSO		*9.7*	*3.8*		14, 7, 8
TA98	**S-8 (POSF4734)**	0.156 µl	*34.3*	*4.0*	*0.9*	30, 38, 35
		0.313 µl	*31.3*	*4.9*	*0.8*	37, 28, 29
		0.625 µl	*34.7*	*3.1*	*0.9*	38, 34, 32
		1.25 µl	*37.7*	*6.5*	*1.0*	31, 44, 38
		2.5 µl	*33.7*	*3.1*	*0.9*	33, 31, 37
		5 µl	*36.7*	*3.8*	*1.0*	41 N, 35 N, 34 N
	DMSO		*37.0*	*2.6*		38, 34, 39
TA100	**S-8 (POSF4734)**	0.156 µl	*142.7*	*3.5*	*1.2*	146, 139, 143
		0.313 µl	*125.7*	*16.7*	*1.0*	116, 145, 116
		0.625 µl	*146.7*	*11.4*	*1.2*	156, 134, 150
		1.25 µl	*128.3*	*5.1*	*1.0*	134, 127, 124
		2.5 µl	*125.3*	*5.7*	*1.0*	127, 130, 119
		5 µl	*118.3*	*6.0*	*1.0*	112 N, 119 N, 124 N
	DMSO		*123.3*	*21.9*		136, 98, 136
WP2uvrA	**S-8 (POSF4734)**	0.156 µl	*33.3*	*4.0*	*1.0*	29, 37, 34
		0.313 µl	*41.7*	*3.5*	*1.2*	42, 38, 45
		0.625 µl	*34.3*	*3.2*	*1.0*	32, 38, 33
		1.25 µl	*39.7*	*4.5*	*1.2*	35, 40, 44
		2.5 µl	*40.0*	*3.5*	*1.2*	38, 44, 38
		5 µl	*31.0*	*9.2*	*0.9*	41 N, 29 N, 23 N
	DMSO		*33.3*	*5.9*		29, 31, 40

Key to Plate Postfix Codes

N Normal background lawn

APPENDIX G. INDIVIDUAL AND MEAN PLATE COUNTS: SECOND MUTAGENICITY EXPERIMENT WITH FIVE JET FUELS

Table G-1. Amyris plate counts

Study Name: G343-10 (Amyris)
Experiment: 2nd Mutagenicity **Positive Controls**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Amyris)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**SA**	5 µg	*2138.7*	*43.0*	*128.3*	2181, 2140, 2095
TA1537	**9AA**	50 µg	*390.3*	*147.5*	*27.2*	243, 390, 538
TA98	**2NF**	5 µg	*1038.3*	*20.3*	*44.5*	1061, 1022, 1032
TA100	**SA**	5 µg	*2075.3*	*194.6*	*15.2*	1938, 1990, 2298
WP2uvrA	**4NQO**	2.5 µg	*1483.3*	*149.5*	*62.7*	1503, 1622, 1325
TA1535	**DMSO**	--	*14.3*	*7.8*	*0.9*	23, 8, 12
TA1537	**DMSO**	--	*13.0*	*2.6*	*0.9*	11, 12, 16
TA98	**DMSO**	--	*22.3*	*7.5*	*1.0*	15, 30, 22
TA100	**DMSO**	--	*136.3*	*11.6*	*1.0*	124, 147, 138
WP2uvrA	**DMSO**	--	*34.0*	*7.9*	*1.4*	40, 25, 37

Key to Positive Controls

SA	Sodium Azide
9AA	9-Aminoacridine hydrochloride
2NF	2-Nitrofluorene
4NQO	4-Nitroquinoline N-oxide
DMSO	Dimethyl Sulfoxide

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

With metabolic activation (10% S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**2AN (10% S9)**	4 µg	*332.3*	*2.1*	*18.1*	330, 334, 333
TA1537	**2AN (10% S9)**	4 µg	*556.0*	*40.0*	*47.7*	596, 556, 516
TA98	**2AN (10% S9)**	2 µg	*2110.0*	*105.7*	*68.1*	2225, 2088, 2017
TA100	**2AN (10% S9)**	2 µg	*2494.7*	*191.1*	*17.0*	2550, 2652, 2282
WP2uvrA	**2AN (10% S9)**	20 µg	*360.7*	*81.5*	*9.1*	388, 425, 269
TA1535	**DMSO (+S9)**	--	*10.3*	*0.6*	*0.6*	11, 10, 10
TA1537	**DMSO (+S9)**	--	*15.0*	*4.0*	*1.3*	19, 15, 11
TA98	**DMSO (+S9)**	--	*33.3*	*5.9*	*1.1*	31, 29, 40
TA100	**DMSO (+S9)**	--	*145.3*	*11.7*	*1.0*	135, 158, 143
WP2uvrA	**DMSO (+S9)**	--	*37.0*	*2.0*	*0.9*	37, 35, 39

Key to Positive Controls

2AN (10% S9)	2-Aminoanthracene (10% S9)
DMSO (+S9)	Dimethyl Sulfoxide +S9

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table G-1 (continued). Amyris plate counts

Study Name: G343-10 (Amyris)
Experiment: 2nd Mutagenicity G343-10 **(Amyris POSF5630)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Amyris)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**Amyris (POSF5630)**	0.0013 µl	*15.3*	*5.0*	*0.9*	20, 16, 10
		0.0025 µl	*13.0*	*5.2*	*0.8*	16, 7, 16
		0.005 µl	*13.7*	*2.5*	*0.8*	16, 11, 14
		0.01 µl	*11.0*	*3.6*	*0.7*	12 N, 14 N, 7 N
		0.02 µl	*8.0*	*1.7*	*0.5*	10 H, 7 H, 7 H
		0.039 µl	*10.7*	*2.5*	*0.6*	11 H, 13 H, 8 H
		0.078 µl	*7.7*	*1.5*	*0.5*	9 H, 6 H, 8 H
	DMSO		*16.7*	*2.1*		19, 15, 16
	Untreated Control		*13.4*	*4.0*		14, 10, 20, 11, 12
TA1537	**Amyris (POSF5630)**	0.0013 µl	*11.0*	*3.0*	*0.8*	8, 11, 14
		0.0025 µl	*11.7*	*4.0*	*0.8*	8, 11, 16
		0.005 µl	*10.7*	*1.2*	*0.7*	12, 10, 10
		0.01 µl	*7.3*	*2.1*	*0.5*	5, 8, 9
		0.02 µl	*7.0*	*2.6*	*0.5*	6 N, 5 N, 10 N
		0.039 µl	*6.7*	*2.5*	*0.5*	4 H, 9 H, 7 H
		0.078 µl	*4.3*	*1.2*	*0.3*	3 H, 5 H, 5 H
	DMSO		*14.3*	*0.6*		14, 14, 15
	Untreated Control		*12.0*	*2.3*		15, 10, 11, 10, 14
TA98	**Amyris (POSF5630)**	0.0013 µl	*26.0*	*7.0*	*1.1*	19, 26, 33
		0.0025 µl	*22.3*	*4.0*	*1.0*	18, 26, 23
		0.005 µl	*16.0*	*5.6*	*0.7*	15, 11, 22
		0.01 µl	*23.3*	*2.3*	*1.0*	22 N, 26 N, 22 N
		0.02 µl	*18.0*	*3.5*	*0.8*	20 H, 20 H, 14 H
		0.039 µl	*20.3*	*5.0*	*0.9*	25 H, 21 H, 15 H
		0.078 µl	*12.0*	*3.6*	*0.5*	11 H, 16 H, 9 H
	DMSO		*23.3*	*1.5*		22, 23, 25
	Untreated Control		*24.6*	*5.9*		15, 23, 29, 27, 29
TA100	**Amyris (POSF5630)**	0.0013 µl	*159.0*	*13.9*	*1.2*	152, 175, 150
		0.0025 µl	*155.3*	*11.7*	*1.1*	142, 164, 160
		0.005 µl	*139.0*	*14.7*	*1.0*	156, 130, 131
		0.01 µl	*142.0*	*12.0*	*1.0*	154, 142, 130
		0.02 µl	*100.7*	*10.2*	*0.7*	89 N, 105 N, 108 N
		0.039 µl	*96.0*	*18.7*	*0.7*	111 H, 75 H, 102 H
		0.078 µl	*88.0*	*9.2*	*0.6*	96 H, 90 H, 78 H
	DMSO		*136.7*	*26.3*		120, 123, 167
	Untreated Control		*122.0*	*13.7*		135, 126, 109, 134, 106
WP2uvrA	**Amyris (POSF5630)**	0.078 µl	*28.3*	*4.2*	*1.2*	25, 33, 27
		0.156 µl	*32.3*	*1.2*	*1.4*	33, 33, 31
		0.313 µl	*24.7*	*1.5*	*1.0*	23, 26, 25
		0.625 µl	*26.3*	*3.1*	*1.1*	27, 29, 23
		1.25 µl	*22.3*	*2.5*	*0.9*	22, 20, 25
		2.5 µl	*24.3*	*2.1*	*1.0*	22 N, 25 N, 26 N
		5 µl	*22.0*	*4.4*	*0.9*	27 H, 19 H, 20 H
	DMSO		*23.7*	*7.8*		30, 26, 15
	Untreated Control		*39.6*	*7.9*		33, 49, 44, 30, 42

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table G-1 (continued). Amyris plate counts

Study Name: G343-10 (Amyris)
Experiment: 2nd Mutagenicity G343-10 **(Amyris POSF5630)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Amyris)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

With metabolic activation (10%S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**Amyris (POSF5630)**	0.0013 µl	*11.0*	*3.6*	*0.6*	12, 14, 7
		0.0025 µl	*12.7*	*2.3*	*0.7*	14, 10, 14
		0.005 µl	*11.3*	*1.2*	*0.6*	10, 12, 12
		0.01 µl	*12.0*	*2.6*	*0.7*	11, 15, 10
		0.02 µl	*11.3*	*1.5*	*0.6*	11 N, 13 N, 10 N
		0.039 µl	*11.0*	*1.0*	*0.6*	11 H, 12 H, 10 H
		0.078 µl	*9.0*	*1.0*	*0.5*	9 H, 10 H, 8 H
	DMSO		*18.3*	*3.8*		20, 21, 14
TA1537	**Amyris (POSF5630)**	0.0013 µl	*9.0*	*3.6*	*0.8*	12, 10, 5
		0.0025 µl	*8.3*	*1.5*	*0.7*	8, 10, 7
		0.005 µl	*9.0*	*2.6*	*0.8*	10, 6, 11
		0.01 µl	*11.3*	*2.3*	*1.0*	10, 10, 14
		0.02 µl	*13.3*	*1.2*	*1.1*	14 N, 14 N, 12 N
		0.039 µl	*8.3*	*4.7*	*0.7*	3 H, 12 H, 10 H
		0.078 µl	*8.3*	*3.5*	*0.7*	5 H, 8 H, 12 H
	DMSO		*11.7*	*0.6*		12, 11, 12
TA98	**Amyris (POSF5630)**	0.0013 µl	*24.7*	*2.3*	*0.8*	22, 26, 26
		0.0025 µl	*32.7*	*3.1*	*1.1*	32, 36, 30
		0.005 µl	*33.3*	*8.5*	*1.1*	33, 25, 42
		0.01 µl	*28.0*	*2.6*	*0.9*	30, 29, 25
		0.02 µl	*34.3*	*3.5*	*1.1*	31 N, 38 N, 34 N
		0.039 µl	*25.3*	*5.5*	*0.8*	20 H, 25 H, 31 H
		0.078 µl	*30.3*	*3.8*	*1.0*	32 H, 33 H, 26 H
	DMSO		*31.0*	*5.2*		34, 25, 34
TA100	**Amyris (POSF5630)**	0.0013 µl	*121.3*	*5.7*	*0.8*	115, 123, 126
		0.0025 µl	*117.3*	*13.1*	*0.8*	131, 105, 116
		0.005 µl	*110.7*	*5.9*	*0.8*	115, 104, 113
		0.01 µl	*122.0*	*8.7*	*0.8*	117, 132, 117
		0.02 µl	*136.7*	*7.5*	*0.9*	141, 128, 141
		0.039 µl	*133.3*	*2.1*	*0.9*	131 N, 134 N, 135 N
		0.078 µl	*107.0*	*5.6*	*0.7*	101 H, 108 H, 112 H
	DMSO		*147.0*	*8.2*		138, 154, 149
WP2uvrA	**Amyris (POSF5630)**	0.078 µl	*39.7*	*11.0*	*1.0*	46, 46, 27
		0.156 µl	*42.0*	*0.0*	*1.1*	42, 42, 42
		0.313 µl	*31.7*	*11.2*	*0.8*	29, 44, 22
		0.625 µl	*36.0*	*5.3*	*0.9*	38, 40, 30
		1.25 µl	*30.3*	*3.5*	*0.8*	30, 34, 27
		2.5 µl	*31.3*	*6.1*	*0.8*	38, 26, 30
		5 µl	*20.7*	*2.3*	*0.5*	22 N, 22 N, 18 N
	DMSO		*39.7*	*6.8*		32, 42, 45

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table G-2. R-8 plate counts

Study Name: G343-10 (R-8)
Experiment: 2nd Mutagenicity G343-10 **(R-8 POSF5469)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**R-8 (POSF5469)**	0.156 µl	*16.3*	*5.1*	*1.0*	15, 12, 22
		0.313 µl	*15.3*	*3.1*	*0.9*	18, 12, 16
		0.625 µl	*18.7*	*8.1*	*1.1*	10, 20, 26
		1.25 µl	*15.0*	*1.0*	*0.9*	15, 14, 16
		2.5 µl	*14.0*	*2.0*	*0.8*	14, 16, 12
		5 µl	*19.7*	*2.9*	*1.2*	18 N, 23 N, 18 N
	DMSO		*16.7*	*2.1*		19, 15, 16
	Untreated Control		*13.4*	*4.0*		14, 10, 20, 11, 12
TA1537	**R-8 (POSF5469)**	0.156 µl	*12.3*	*4.0*	*0.9*	13, 16, 8
		0.313 µl	*12.0*	*2.6*	*0.8*	15, 10, 11
		0.625 µl	*12.0*	*1.0*	*0.8*	11, 13, 12
		1.25 µl	*16.0*	*2.6*	*1.1*	14, 19, 15
		2.5 µl	*15.0*	*3.6*	*1.0*	11, 16, 18
		5 µl	*17.0*	*1.7*	*1.2*	15 N, 18 N, 18 N
	DMSO		*14.3*	*0.6*		14, 14, 15
	Untreated Control		*12.0*	*2.3*		15, 10, 11, 10, 14
TA98	**R-8 (POSF5469)**	0.156 µl	*21.0*	*5.6*	*0.9*	22, 15, 26
		0.313 µl	*30.0*	*3.6*	*1.3*	29, 34, 27
		0.625 µl	*25.3*	*3.5*	*1.1*	29, 25, 22
		1.25 µl	*21.0*	*3.6*	*0.9*	18, 25, 20
		2.5 µl	*23.0*	*3.0*	*1.0*	23, 26, 20
		5 µl	*26.7*	*9.1*	*1.1*	23 N, 37 N, 20 N
	DMSO		*23.3*	*1.5*		22, 23, 25
	Untreated Control		*24.6*	*5.9*		15, 23, 29, 27, 29
TA100	**R-8 (POSF5469)**	0.156 µl	*114.0*	*5.6*	*0.8*	108, 119, 115
		0.313 µl	*106.7*	*15.1*	*0.8*	96, 124, 100
		0.625 µl	*107.0*	*5.2*	*0.8*	104, 113, 104
		1.25 µl	*110.0*	*7.5*	*0.8*	111, 102, 117
		2.5 µl	*115.3*	*18.4*	*0.8*	95, 131, 120
		5 µl	*97.0*	*15.6*	*0.7*	89 N, 87 N, 115 N
	DMSO		*136.7*	*26.3*		120, 123, 167
	Untreated Control		*122.0*	*13.7*		135, 126, 109, 134, 106
WP2uvrA	**R-8 (POSF5469)**	0.156 µl	*24.0*	*5.3*	*1.0*	22, 20, 30
		0.313 µl	*32.0*	*2.6*	*1.4*	31, 30, 35
		0.625 µl	*26.3*	*6.7*	*1.1*	22, 34, 23
		1.25 µl	*33.3*	*5.7*	*1.4*	38, 35, 27
		2.5 µl	*21.3*	*7.1*	*0.9*	29, 20, 15
		5 µl	*24.0*	*6.9*	*1.0*	32 N, 20 N, 20 N
	DMSO		*23.7*	*7.8*		30, 26, 15
	Untreated Control		*39.6*	*7.9*		33, 49, 44, 30, 42

Key to Plate Postfix Codes

N Normal background lawn

Table G-2 (continued). R-8 plate counts

Study Name: G343-10 (R-8)
Experiment: 2nd Mutagenicity G343-10 **(R-8 POSF5469)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

With metabolic activation (10%S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**R-8 (POSF5469)**	0.156 µl	*19.3*	*7.5*	*1.1*	27, 19, 12
		0.313 µl	*15.7*	*3.5*	*0.9*	16, 12, 19
		0.625 µl	*19.3*	*3.1*	*1.1*	22, 16, 20
		1.25 µl	*12.0*	*5.6*	*0.7*	11, 7, 18
		2.5 µl	*12.3*	*2.3*	*0.7*	15, 11, 11
		5 µl	*18.7*	*6.5*	*1.0*	12 N, 19 N, 25 N
	DMSO		*18.3*	*3.8*		20, 21, 14
TA1537	**R-8 (POSF5469)**	0.156 µl	*16.0*	*2.0*	*1.4*	14, 18, 16
		0.313 µl	*16.0*	*3.0*	*1.4*	13, 19, 16
		0.625 µl	*14.3*	*2.1*	*1.2*	15, 12, 16
		1.25 µl	*10.0*	*2.6*	*0.9*	7, 11, 12
		2.5 µl	*12.7*	*3.1*	*1.1*	10, 12, 16
		5 µl	*14.7*	*3.1*	*1.3*	18 N, 14 N, 12 N
	DMSO		*11.7*	*0.6*		12, 11, 12
TA98	**R-8 (POSF5469)**	0.156 µl	*31.0*	*4.0*	*1.0*	35, 27, 31
		0.313 µl	*35.0*	*5.6*	*1.1*	40, 29, 36
		0.625 µl	*29.3*	*7.5*	*0.9*	38, 25, 25
		1.25 µl	*29.7*	*6.7*	*1.0*	22, 33, 34
		2.5 µl	*37.0*	*1.0*	*1.2*	36, 38, 37
		5 µl	*32.7*	*1.2*	*1.1*	34 N, 32 N, 32 N
	DMSO		*31.0*	*5.2*		34, 25, 34
TA100	**R-8 (POSF5469)**	0.156 µl	*157.0*	*9.6*	*1.1*	161, 164, 146
		0.313 µl	*152.3*	*7.5*	*1.0*	160, 145, 152
		0.625 µl	*144.3*	*24.5*	*1.0*	119, 146, 168
		1.25 µl	*131.3*	*10.0*	*0.9*	132, 141, 121
		2.5 µl	*129.7*	*12.5*	*0.9*	130, 142, 117
		5 µl	*130.0*	*20.8*	*0.9*	119 N, 117 N, 154 N
	DMSO		*147.0*	*8.2*		138, 154, 149
WP2uvrA	**R-8 (POSF5469)**	0.156 µl	*31.3*	*2.5*	*0.8*	31, 29, 34
		0.313 µl	*34.0*	*3.0*	*0.9*	34, 31, 37
		0.625 µl	*32.0*	*7.9*	*0.8*	23, 35, 38
		1.25 µl	*35.7*	*7.1*	*0.9*	37, 42, 28
		2.5 µl	*31.3*	*7.6*	*0.8*	33, 23, 38
		5 µl	*36.3*	*9.0*	*0.9*	45 N, 27 N, 37 N
	DMSO		*39.7*	*6.8*		32, 42, 45

Key to Plate Postfix Codes

N Normal background lawn

Table G-3. R-8 from algae plate counts

Study Name: G343-10 (R-8 algae)
Experiment: 2nd Mutagenicity G343-10 **(R-8 from algae POSF5804)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8 algae)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**R-8 from algae (POSF5804)**	0.156 µl	*14.7*	*1.2*	*0.9*	16, 14, 14
		0.313 µl	*15.0*	*6.1*	*0.9*	11, 12, 22
		0.625 µl	*11.7*	*7.6*	*0.7*	10, 5, 20
		1.25 µl	*12.7*	*5.0*	*0.8*	12, 18, 8
		2.5 µl	*8.0*	*0.0*	*0.5*	8, 8, 8
		5 µl	*9.7*	*4.5*	*0.6*	10 P N, 5 P N, 14 P N
	DMSO		*16.7*	*2.1*		19, 15, 16
	Untreated Control		*13.4*	*4.0*		14, 10, 20, 11, 12
TA1537	**R-8 from algae (POSF5804)**	0.156 µl	*12.0*	*2.0*	*0.8*	14, 12, 10
		0.313 µl	*13.7*	*5.1*	*1.0*	18, 15, 8
		0.625 µl	*12.0*	*4.4*	*0.8*	14, 15, 7
		1.25 µl	*10.7*	*1.2*	*0.7*	10, 10, 12
		2.5 µl	*4.7*	*2.1*	*0.3*	4, 3, 7
		5 µl	*7.3*	*2.5*	*0.5*	10 P N, 5 P N, 7 P N
	DMSO		*14.3*	*0.6*		14, 14, 15
	Untreated Control		*12.0*	*2.3*		15, 10, 11, 10, 14
TA98	**R-8 from algae (POSF5804)**	0.156 µl	*21.3*	*2.1*	*0.9*	22, 19, 23
		0.313 µl	*22.3*	*5.8*	*1.0*	29, 19, 19
		0.625 µl	*24.7*	*9.0*	*1.1*	19, 20, 35
		1.25 µl	*15.3*	*3.1*	*0.7*	16, 18, 12
		2.5 µl	*18.3*	*0.6*	*0.8*	18, 19, 18
		5 µl	*22.0*	*6.1*	*0.9*	15 P N, 25 P N, 26 P N
	DMSO		*23.3*	*1.5*		22, 23, 25
	Untreated Control		*24.6*	*5.9*		15, 23, 29, 27, 29
TA100	**R-8 from algae (POSF5804)**	0.156 µl	*111.0*	*9.0*	*0.8*	120, 111, 102
		0.313 µl	*108.7*	*2.5*	*0.8*	111, 106, 109
		0.625 µl	*110.0*	*16.1*	*0.8*	105, 97, 128
		1.25 µl	*103.3*	*13.0*	*0.8*	104, 90, 116
		2.5 µl	*95.7*	*7.4*	*0.7*	93, 90, 104
		5 µl	*99.0*	*1.7*	*0.7*	98 P N, 98 P N, 101 P N
	DMSO		*136.7*	*26.3*		120, 123, 167
	Untreated Control		*122.0*	*13.7*		135, 126, 109, 134, 106
WP2uvrA	**R-8 from algae (POSF5804)**	0.156 µl	*32.7*	*6.8*	*1.4*	38, 35, 25
		0.313 µl	*22.3*	*3.5*	*0.9*	19, 22, 26
		0.625 µl	*28.3*	*2.1*	*1.2*	26, 29, 30
		1.25 µl	*26.0*	*3.6*	*1.1*	23, 30, 25
		2.5 µl	*31.7*	*6.1*	*1.3*	25, 33, 37
		5 µl	*29.0*	*7.2*	*1.2*	37 P N, 27 P N, 23 P N
	DMSO		*23.7*	*7.8*		30, 26, 15
	Untreated Control		*39.6*	*7.9*		33, 49, 44, 30, 42

Key to Plate Postfix Codes

P	Precipitate seen as oil like droplets
N	Normal background lawn

Table G-3 (continued). R-8 from algae plate counts

Study Name: G343-10 (R-8 algae)
Experiment: 2nd Mutagenicity G343-10 **(R-8 from algae POSF5804)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (R-8 algae)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

With metabolic activation (10%S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	R-8 from algae (POSF5804)	0.156 µl	*13.7*	*1.5*	*0.7*	15, 12, 14
		0.313 µl	*11.7*	*3.5*	*0.6*	12, 15, 8
		0.625 µl	*13.0*	*1.7*	*0.7*	14, 11, 14
		1.25 µl	*10.0*	*5.6*	*0.5*	16, 5, 9
		2.5 µl	*13.7*	*1.5*	*0.7*	12, 14, 15
		5 µl	*12.0*	*1.7*	*0.7*	14 P N, 11 P N, 11 P N
	DMSO		*18.3*	*3.8*		20, 21, 14
TA1537	R-8 from algae (POSF5804)	0.156 µl	*10.3*	*4.7*	*0.9*	12, 5, 14
		0.313 µl	*16.3*	*2.3*	*1.4*	15, 19, 15
		0.625 µl	*16.0*	*1.0*	*1.4*	15, 16, 17
		1.25 µl	*14.0*	*4.4*	*1.2*	12, 11, 19
		2.5 µl	*12.7*	*1.2*	*1.1*	12, 12, 14
		5 µl	*14.0*	*1.7*	*1.2*	15 P N, 15 P N, 12 P N
	DMSO		*11.7*	*0.6*		12, 11, 12
TA98	R-8 from algae (POSF5804)	0.156 µl	*29.7*	*1.2*	*1.0*	29, 29, 31
		0.313 µl	*26.3*	*4.2*	*0.8*	25, 23, 31
		0.625 µl	*30.0*	*4.6*	*1.0*	29, 26, 35
		1.25 µl	*33.7*	*4.5*	*1.1*	34, 38, 29
		2.5 µl	*26.7*	*4.5*	*0.9*	22, 27, 31
		5 µl	*31.3*	*8.3*	*1.0*	34 P N, 22 P N, 38 P N
	DMSO		*31.0*	*5.2*		34, 25, 34
TA100	R-8 from algae (POSF5804)	0.156 µl	*126.0*	*7.2*	*0.9*	124, 120, 134
		0.313 µl	*138.7*	*9.3*	*0.9*	145, 128, 143
		0.625 µl	*135.3*	*28.9*	*0.9*	104, 161, 141
		1.25 µl	*127.3*	*18.9*	*0.9*	142, 134, 106
		2.5 µl	*145.7*	*11.1*	*1.0*	156, 134, 147
		5 µl	*119.0*	*7.5*	*0.8*	126 P N, 120 P N, 111 P N
	DMSO		*147.0*	*8.2*		138, 154, 149
WP2uvrA	R-8 from algae (POSF5804)	0.156 µl	*38.3*	*2.3*	*1.0*	37, 37, 41
		0.313 µl	*30.3*	*4.0*	*0.8*	31, 34, 26
		0.625 µl	*38.7*	*3.2*	*1.0*	41, 40, 35
		1.25 µl	*34.0*	*8.0*	*0.9*	26, 42, 34
		2.5 µl	*38.3*	*9.9*	*1.0*	27, 45, 43
		5 µl	*39.3*	*4.5*	*1.0*	44 P N, 35 P N, 39 P N
	DMSO		*39.7*	*6.8*		32, 42, 45

Key to Plate Postfix Codes

P	Precipitate seen as oil like droplets
N	Normal background lawn

Table G-4. S-8 plate counts

Study Name: G343-10 (S-8)
Experiment: 2nd Mutagenicity **(S-8 POSF4734)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (S-8)
Date Plated: 4/2/2010
Date Counted: 3/25/2010 to 4/9/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	**S-8 (POSF4734)**	0.156 µl	*15.7*	*2.1*	*1.7*	18, 14, 15
		0.313 µl	*16.7*	*7.4*	*1.8*	25, 14, 11
		0.625 µl	*12.7*	*4.2*	*1.4*	16, 8, 14
		1.25 µl	*15.3*	*2.3*	*1.6*	14, 18, 14
		2.5 µl	*14.7*	*2.3*	*1.6*	16, 12, 16
		5 µl	*14.0*	*3.5*	*1.5*	16 N, 10 N, 16 N
	DMSO		*9.3*	*2.5*		7, 12, 9
	Untreated Control		*8.6*	*2.7*		7, 12, 9, 10, 5
TA1537	**S-8 (POSF4734)**	0.156 µl	*16.0*	*3.0*	*1.1*	19, 16, 13
		0.313 µl	*12.3*	*5.5*	*0.9*	12, 7, 18
		0.625 µl	*9.0*	*1.7*	*0.6*	10, 7, 10
		1.25 µl	*12.3*	*1.5*	*0.9*	11, 12, 14
		2.5 µl	*15.0*	*3.6*	*1.0*	18, 16, 11
		5 µl	*10.0*	*2.6*	*0.7*	7 N, 12 N, 11 N
	DMSO		*14.3*	*0.6*		14, 14, 15
	Untreated Control		*12.0*	*2.3*		15, 10, 11, 10, 14
TA98	**S-8 (POSF4734)**	0.156 µl	*25.0*	*4.6*	*1.1*	26, 20, 29
		0.313 µl	*20.3*	*4.5*	*0.9*	16, 20, 25
		0.625 µl	*18.7*	*3.5*	*0.8*	19, 15, 22
		1.25 µl	*25.3*	*5.7*	*1.1*	19, 27, 30
		2.5 µl	*20.0*	*3.5*	*0.9*	22, 22, 16
		5 µl	*21.0*	*3.6*	*0.9*	25 N, 20 N, 18 N
	DMSO		*23.3*	*1.5*		22, 23, 25
	Untreated Control		*24.6*	*5.9*		15, 23, 29, 27, 29
TA100	**S-8 (POSF4734)**	0.156 µl	*116.0*	*18.0*	*0.8*	98, 116, 134
		0.313 µl	*125.0*	*16.5*	*0.9*	106, 135, 134
		0.625 µl	*124.7*	*13.0*	*0.9*	124, 112, 138
		1.25 µl	*121.3*	*17.5*	*0.9*	136, 126, 102
		2.5 µl	*117.0*	*16.8*	*0.9*	111, 104, 136
		5 µl	*98.0*	*11.8*	*0.7*	85 N, 108 N, 101 N
	DMSO		*136.7*	*26.3*		120, 123, 167
	Untreated Control		*122.0*	*13.7*		135, 126, 109, 134, 106
WP2uvrA	**S-8 (POSF4734)**	0.156 µl	*27.7*	*7.6*	*1.2*	33, 31, 19
		0.313 µl	*26.7*	*5.7*	*1.1*	33, 22, 25
		0.625 µl	*30.7*	*4.5*	*1.3*	26, 31, 35
		1.25 µl	*32.3*	*4.2*	*1.4*	29, 31, 37
		2.5 µl	*26.3*	*11.8*	*1.1*	19, 20, 40
		5 µl	*34.3*	*3.1*	*1.5*	37 N, 35 N, 31 N
	DMSO		*23.7*	*7.8*		30, 26, 15
	Untreated Control		*39.6*	*7.9*		33, 49, 44, 30, 42

Key to Plate Postfix Codes

N Normal background lawn

Table G-4 (continued). S-8 plate counts

Study Name: G343-10 (S-8)
Experiment: 2nd Mutagenicity (S-8 POSF4734)
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (S-8)
Date Plated: 4/2/2010
Date Counted: 3/25/2010 to 4/9/2010

With metabolic activation (10%S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	S-8 (POSF4734)	0.156 µl	15.0	4.0	1.1	15, 11, 19
		0.313 µl	12.7	5.0	0.9	8, 18, 12
		0.625 µl	11.7	2.9	0.9	15, 10, 10
		1.25 µl	15.0	6.1	1.1	12, 11, 22
		2.5 µl	13.3	1.2	1.0	12, 14, 14
		5 µl	10.3	3.2	0.8	8 N, 14 N, 9 N
	DMSO		13.7	3.5		10, 14, 17
TA1537	S-8 (POSF4734)	0.156 µl	11.3	3.1	1.0	12, 14, 8
		0.313 µl	13.3	4.2	1.1	10, 12, 18
		0.625 µl	12.7	2.1	1.1	15, 12, 11
		1.25 µl	14.0	2.0	1.2	16, 14, 12
		2.5 µl	13.3	1.2	1.1	14, 14, 12
		5 µl	16.3	4.6	1.4	11 N, 19 N, 19 N
	DMSO		11.7	0.6		12, 11, 12
TA98	S-8 (POSF4734)	0.156 µl	34.0	4.0	1.1	30, 34, 38
		0.313 µl	37.0	4.0	1.2	41, 33, 37
		0.625 µl	36.3	7.6	1.2	31, 45, 33
		1.25 µl	36.0	10.1	1.2	25, 38, 45
		2.5 µl	33.3	4.0	1.1	31, 38, 31
		5 µl	40.0	2.0	1.3	38 N, 42 N, 40 N
	DMSO		31.0	5.2		34, 25, 34
TA100	S-8 (POSF4734)	0.156 µl	148.3	7.1	1.0	147, 156, 142
		0.313 µl	160.3	16.8	1.1	175, 164, 142
		0.625 µl	155.3	6.1	1.1	162, 150, 154
		1.25 µl	153.3	9.5	1.0	146, 150, 164
		2.5 µl	138.7	22.6	0.9	115, 160, 141
		5 µl	144.3	2.1	1.0	145 N, 146 N, 142 N
	DMSO		147.0	8.2		138, 154, 149
WP2uvrA	S-8 (POSF4734)	0.156 µl	36.3	9.6	0.9	45, 38, 26
		0.313 µl	39.0	8.5	1.0	31, 48, 38
		0.625 µl	32.3	10.8	0.8	40, 20, 37
		1.25 µl	33.3	4.0	0.8	38, 31, 31
		2.5 µl	30.3	5.8	0.8	27, 27, 37
		5 µl	35.0	6.2	0.9	42 N, 33 N, 30 N
	DMSO		39.7	6.8		32, 42, 45

Key to Plate Postfix Codes

N Normal background lawn

Table G-5. Swedish Biofuel plate counts

Study Name: G343-10 (Swedish)
Experiment: 2nd Mutagenicity G343-10 **(Swedish Biofuel POSF5668)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Swedish)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	Swedish Biofuel (POSF5668)	0.156 µl	15.3	4.0	0.9	19, 16, 11
		0.313 µl	13.3	4.6	0.8	16, 8, 16
		0.625 µl	18.3	3.1	1.1	19, 21, 15
		1.25 µl	18.7	3.1	1.1	18, 22, 16
		2.5 µl	13.3	5.1	0.8	9 N, 19 N, 12 N
		5 µl	11.3	3.1	0.7	12 H, 14 H, 8 H
	DMSO		16.7	2.1		19, 15, 16
	Untreated Control		13.4	4.0		14, 10, 20, 11, 12
TA1537	Swedish Biofuel (POSF5668)	0.156 µl	10.7	4.7	0.7	7, 16, 9
		0.313 µl	7.0	3.6	0.5	11, 6, 4
		0.625 µl	10.3	2.1	0.7	12, 11, 8
		1.25 µl	9.7	2.5	0.7	12, 7, 10
		2.5 µl	9.3	2.1	0.7	7 N, 10 N, 11 N
		5 µl	7.0	2.6	0.5	10 H, 5 H, 6 H
	DMSO		14.3	0.6		14, 14, 15
	Untreated Control		12.0	2.3		15, 10, 11, 10, 14
TA98	Swedish Biofuel (POSF5668)	0.156 µl	24.3	6.0	1.0	30, 18, 25
		0.313 µl	25.3	2.1	1.1	23, 27, 26
		0.625 µl	22.7	6.5	1.0	16, 29, 23
		1.25 µl	22.0	7.9	0.9	19, 31, 16
		2.5 µl	22.3	4.0	1.0	27 N, 20 N, 20 N
		5 µl	12.7	3.8	0.5	17 H, 10 H, 11 H
	DMSO		23.3	1.5		22, 23, 25
	Untreated Control		24.6	5.9		15, 23, 29, 27, 29
TA100	Swedish Biofuel (POSF5668)	0.156 µl	114.7	15.7	0.8	127, 97, 120
		0.313 µl	109.0	9.8	0.8	106, 120, 101
		0.625 µl	122.0	19.7	0.9	143, 104, 119
		1.25 µl	154.7	14.0	1.1	141, 169, 154
		2.5 µl	139.0	16.5	1.0	123 N, 156 N, 138 N
		5 µl	117.0	4.6	0.9	113 H, 116 H, 122 H
	DMSO		136.7	26.3		120, 123, 167
	Untreated Control		122.0	13.7		135, 126, 109, 134, 106
WP2uvrA	Swedish Biofuel (POSF5668)	0.156 µl	27.7	3.1	1.2	31, 27, 25
		0.313 µl	25.0	7.9	1.1	19, 34, 22
		0.625 µl	28.0	7.9	1.2	31, 19, 34
		1.25 µl	28.3	5.7	1.2	22, 30, 33
		2.5 µl	27.3	3.2	1.2	25 N, 31 N, 26 N
		5 µl	24.3	5.7	1.0	29 H, 26 H, 18 H
	DMSO		23.7	7.8		30, 26, 15
	Untreated Control		39.6	7.9		33, 49, 44, 30, 42

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

Table G-5 (continued). Swedish Biofuel plate counts

Study Name: G343-10 (Swedish)
Experiment: 2nd Mutagenicity G343-10 **(Swedish Biofuel POSF5668)**
Assay Conditions: Plate incorporation assay

Study Code: G343-10 (Swedish)
Date Plated: 4/2/2010
Date Counted: 4/8/2010 to 4/9/2010

With metabolic activation (10%S-9)

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA1535	Swedish Biofuel (POSF5668)	0.156 µl	*9.0*	*3.6*	*0.5*	10, 12, 5
		0.313 µl	*15.0*	*4.6*	*0.8*	10, 16, 19
		0.625 µl	*9.7*	*1.5*	*0.5*	11, 8, 10
		1.25 µl	*10.7*	*3.1*	*0.6*	10, 8, 14
		2.5 µl	*13.7*	*2.3*	*0.7*	15 N, 11 N, 15 N
		5 µl	*8.7*	*1.2*	*0.5*	8 H, 8 H, 10 H
	DMSO		*18.3*	*3.8*		20, 21, 14
TA1537	Swedish Biofuel (POSF5668)	0.156 µl	*10.3*	*2.1*	*0.9*	8, 12, 11
		0.313 µl	*10.7*	*4.6*	*0.9*	16, 8, 8
		0.625 µl	*10.7*	*3.1*	*0.9*	10, 8, 14
		1.25 µl	*10.3*	*1.5*	*0.9*	12 N, 10 N, 9 N
		2.5 µl	*7.3*	*1.5*	*0.6*	6 H, 7 H, 9 H
		5 µl	*3.3*	*1.5*	*0.3*	5 H, 3 H, 2 H
	DMSO		*11.7*	*0.6*		12, 11, 12
TA98	Swedish Biofuel (POSF5668)	0.156 µl	*31.3*	*4.0*	*1.0*	35, 32, 27
		0.313 µl	*33.0*	*5.3*	*1.1*	27, 35, 37
		0.625 µl	*33.7*	*1.5*	*1.1*	35, 34, 32
		1.25 µl	*35.7*	*4.0*	*1.2*	31, 38, 38
		2.5 µl	*28.0*	*4.4*	*0.9*	30 N, 31 N, 23 N
		5 µl	*27.0*	*7.0*	*0.9*	27 H, 20 H, 34 H
	DMSO		*31.0*	*5.2*		34, 25, 34
TA100	Swedish Biofuel (POSF5668)	0.156 µl	*146.7*	*24.1*	*1.0*	144, 172, 124
		0.313 µl	*133.3*	*4.2*	*0.9*	132, 138, 130
		0.625 µl	*123.0*	*9.8*	*0.8*	115, 120, 134
		1.25 µl	*126.3*	*9.6*	*0.9*	135, 128, 116
		2.5 µl	*138.0*	*8.7*	*0.9*	148 N, 133 N, 133 N
		5 µl	*126.0*	*4.4*	*0.9*	129 H, 121 H, 128 H
	DMSO		*147.0*	*8.2*		138, 154, 149
WP2uvrA	Swedish Biofuel (POSF5668)	0.156 µl	*34.7*	*8.7*	*0.9*	37, 25, 42
		0.313 µl	*31.0*	*3.5*	*0.8*	29, 35, 29
		0.625 µl	*37.0*	*5.2*	*0.9*	40, 31, 40
		1.25 µl	*34.7*	*3.5*	*0.9*	35, 38, 31
		2.5 µl	*33.0*	*5.6*	*0.8*	27 N, 34 N, 38 N
		5 µl	*26.3*	*4.5*	*0.7*	22 H, 31 H, 26 H
	DMSO		*39.7*	*6.8*		32, 42, 45

Key to Plate Postfix Codes

N	Normal background lawn
H	Thinning lawn

LIST OF ABBREVIATIONS

CofA	Certificate of Analysis
DMSO	dimethyl sulfoxide
EPA	Environmental Protection Agency
FT	Fischer Tropsch
GLP	Good Laboratory Practice
MA	metabolic activation
NCIMB	National Collection of Industrial and Marine Bacteria
OPPTS	Office of Prevention, Pesticides and Toxic Substances
UV	ultraviolet